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(54) Title: MEMBRANE TYPE-1 MATRIX METALLOPROTEIN INHIBITORS AND USES THEREOF

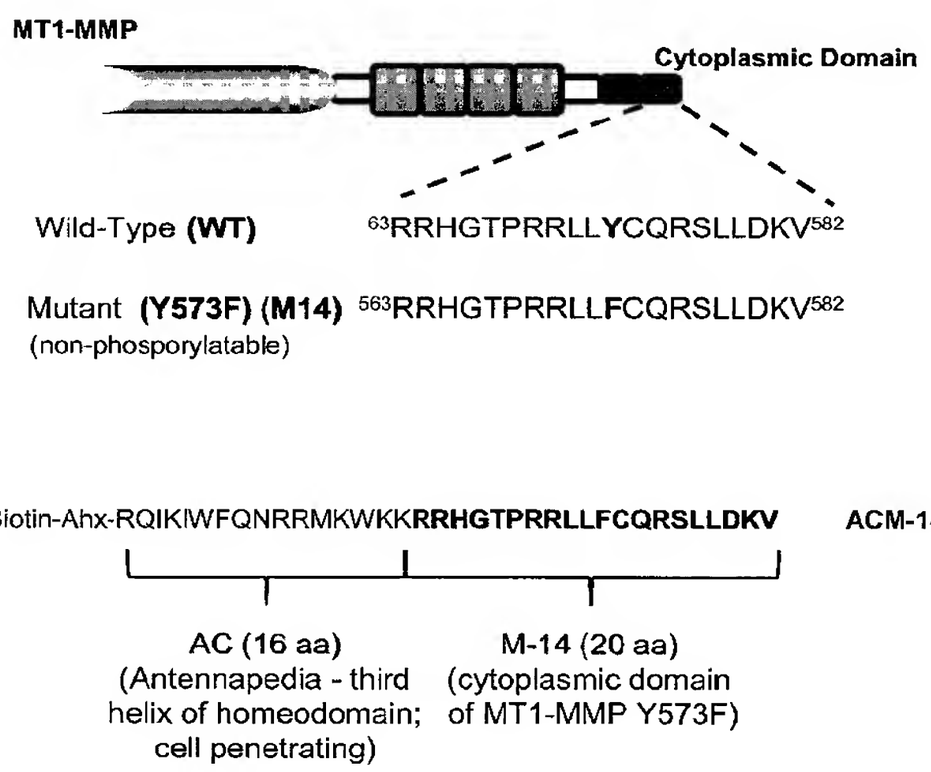

Figure 1

(57) Abstract: Based on the discovery that a soluble polypeptide including a nonphosphorylatable form of the MT1-MMP cytoplasmic domain is capable of inhibiting MT1-MMP in a dominant negative manner, the present invention provides compositions including MT1-MMP inhibitors such as peptide inhibitors, and methods for treating diseases associated with MT1-MMP activity. Such diseases include cancer, arthritis, and heart disease, and vascular disease.

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MEMBRANE TYPE-1 MATRIX METALLOPROTEIN INHIBITORS AND USES THEREOF

Background of the Invention

The invention relates to compounds capable of inhibiting the membrane type-1 metalloproteinase (MT1-MMP or MMP-14), and methods treating diseases such as cancer, heart and vascular disease, and arthritis using these compounds.

Breakdown and remodeling of the extracellular matrix (ECM) has been implicated in disease processes, including cancer, heart and vascular disease, and arthritis.

Cancer is a disease marked by the uncontrolled growth of abnormal cells. Cancer cells have overcome the barriers imposed in normal cells, which have a finite lifespan, to grow indefinitely. As the growth of cancer cells continue, genetic alterations may persist until the cancerous cell manifests an even more aggressive growth phenotype. If left untreated, metastasis, the spread of cancer cells to distant areas of the body by way of the lymph system or bloodstream, may ensue, destroying healthy tissue.

Cancer metastasis requires that the cancer cells leave the original tumor site, usually by entering the blood or lymphatic system, and spread to other regions of the body. Metastatic cells therefore must become free from the tissues in which they originally developed. This process can involve breakdown of ECM structures.

Arthritis also associated with changes in the ECM. In osteoarthritis, degradation of the ECM of articular cartilage occurs, resulting symptoms such as pain, stiffness, limited motion, tenderness, and swelling.

Changes in the ECM are also associated with cardiovascular diseases such as atherosclerosis, especially in the early stages of the disease.

Thus, new approaches to controlling changes in or degradation of the ECM are desirable.

Summary of the Invention

We have made the surprising discovery that a soluble polypeptide containing a nonphosphorylatable form of the cytoplasmic domain of membrane type-1 metalloproteinase (MT1-MMP) is capable of inhibiting MT1-MMP activity and that such inhibition does not require either the transmembrane or extracellular portions of the MT1-MMP sequence. The invention therefore features compositions capable of inhibiting MT1-MMP activity, such as soluble polypeptides including the MT1-MMP cytoplasmic sequence with a mutation or deletion at position 573 of the MT1-MMP sequence. MT1-MMP activity has been associated with collagen degradation, with increased motility and invasiveness of cancer cells, and with diseases such as heart disease, vascular disease, and arthritis. Thus, these compositions can be useful in the treatment of these diseases, or any disease where decreased MT1-MMP activity is desirable. Such compositions may also be administered in conjunction with standard therapeutics used to treat these diseases, as described herein.

Accordingly, in a first aspect, the invention features a composition including a polypeptide (e.g., a soluble polypeptide) including an amino acid sequence substantially identical to the cytoplasmic domain of membrane-type 1 matrix metalloproteinase (MT1-MMP) or a fragment thereof, where the polypeptide is capable of inhibiting (e.g., selectively inhibiting) MT1-MMP activity. The amino acid sequence may lack a phosphorylatable tyrosine at the amino acid position corresponding to position 573 of the human MT1-MMP sequence such as a substitution, deletion, or modification at the position 573. In certain embodiments, the substitution made using a naturally occurring amino acid (e.g., phenylalanine), a non-naturally occurring amino acid, or a modified form thereof. The amino acid sequence may have 0, 1, 2, 3, 4, 5, 6, or 7 substitutions as compared to the corresponding sequence of the

cytoplasmic domain of human MT1-MMP. In a particular embodiment, the amino acid sequence is at least 80% (e.g., 85%, 90%, 95%, or 98%) identical to the sequence of cytoplasmic domain of human MT1-MMP, or the cytoplasmic domain of human MT1-MMP having a deletion, substitution, or modification at the position corresponding to position 573 of the human MT1-MMP sequence (e.g., RRHGTPRRLLFCQRSLLDKV (SEQ ID NO:118) or an MT1-MMP inhibitory fragment thereof).

In other embodiments, the polypeptide includes a delivery vector (e.g., an amino acid sequence capable of penetrating a cellular membrane, capable of entering a particular cell type, or capable of crossing the blood-brain barrier (BBB), such as any of those described herein). The amino acid sequence capable of penetrating a cellular membrane may be substantially identical to a polypeptide selected from the group consisting of the third helix of the homeodomain of the antennapedia protein (SEQ ID NO:119, the antennapedia leader peptide (CT) (SEQ ID NO:120), the antennapedia peptide amide (SEQ ID NO:121), Cys(Npys)-antennapedia peptide amide (SEQ ID NO:122), a cytoplasmic transduction peptide (CTP) (e.g., those described herein), HSV-1 VP22, $(Arg)_9$ (SEQ ID NO:137), Cys(Npys)-$(Arg)_9$ (SEQ ID NO:138), Cys(Npys)-$(D\text{-}Arg)_9$, [Cys58]105Y cell penetrating peptide (SEQ ID NO:139), peptide 105Y (SEQ ID NO:140), buforin (SEQ ID NO:141), chimeric rabies virus glycoprotein fragment (RVG-9R; SEQ ID NO:142), Cys(Npys)-TAT(47-57) (SEQ ID NO:143), Cys-TAT(47-57) (SEQ ID NO:144), lipid membrane translocating peptide (SEQ ID NO:145), D-isomer-lipid membrane translocating peptide, mastoparan (SEQ ID NO:146), mastoparan 7 (SEQ ID NO:147), mastoparan X (SEQ ID NO:148), MEK1 derived peptide inhibitor 1 (SEQ ID NO:149), myristoyl-MEK1 derived peptide inhibitor 1 (SEQ ID NO:150), stearyl-MEK-1 derived peptide inhibitor 1 amide (SEQ ID NO:151), membrane-permeable sequence (SEQ ID NO:152), HIV related MPG ((SEQ ID NO:153), aminopeptidase N ligand (CD13), NGR peptide (SEQ ID NO:154), NGR peptide 1, NGR peptide 2 (SEQ ID NO:155), NGR peptide 3 (SEQ ID NO:156), NGR peptide 4, Pep-1 (Chariot™; SEQ ID NO:157),

SynB1 (SEQ ID NO:158), biotin-TAT(47-57) (SEQ ID NO:159), TAT(47-57) (SEQ ID NO:160), TAT(47-57) GGG-Cys(Npys) (SEQ ID NO:161), TAT(48-57) (SEQ ID NO:162), Tat-C(48-57) (SEQ ID NO:163), transdermal peptide (SEQ ID NO:164), transportan (SEQ ID NO:165), and transportan 10 (SEQ ID NO:166). In certain embodiments, the polypeptide includes the sequences RQIKIWFQNRRMKWKK (SEQ ID NO:119) and RRHGTPRRLLFCQRSLLDKV (SEQ ID NO:118) (e.g., the sequence RQIKIWFQNRRMKWKKRRHGTPRRLLFCQRSLLDKV (SEQ ID NO:176)). In certain embodiments, the amino acid sequence capable of crossing the BBB is an antibody or is at least 90% identical (e.g., 100% identical) to Angiopep-2 (SEQ ID NO:97) or Angiopep-1 (SEQ ID NO:67). The polypeptide may include both Angiopep-2 (SEQ ID NO:97) and the sequence RRHGTPRRLLFCQRSLLDKV (SEQ ID NO:118) (e.g., the sequence TFFYGGSRGKRNNFKTEEYRRHGTPRRLLFCQRSLLDKV (SEQ ID NO:178)). In still other embodiments, the amino acid sequence capable of entering a particular cell type is at least 90% identical (e.g., 100% identical) to Angiopep-7 (SEQ ID NO:112).

The composition may be formulated as a liposomal formulation. The liposome may include a delivery vector (e.g., any described herein). The delivery vector may be on the exterior surface of the liposome.

Any of the compositions may be formulated with a pharmaceutically acceptable carrier (e.g., any of those described herein).

In another aspect, the invention features a method of reducing MT1-MMP phosphorylation in a cell. The method includes administering a composition of the above aspect (e.g., any composition described herein) to the cell. The cell may be in a subject (e.g., a human).

In another aspect, the invention features a method of treating (e.g., prophylactically) a disease characterized by increased MT1-MMP activity. The method includes administering a composition of the first aspect (e.g., any composition described herein) to the subject in an amount sufficient to treat the disease (e.g., cancer, heart or vascular disease, or arthritis).

In another aspect, the invention features a method of treating (e.g., prophylactically) a subject having a cancer. The method includes administering a composition of the first aspect (e.g., any composition described herein) to the subject in an amount sufficient to treat the cancer (e.g., a cancer selected from the group consisting of brain cancer, acute leukemia, acute lymphocytic leukemia, acute myelocytic leukemia, acute myeloblastic leukemia, acute promyelocytic leukemia, acute myelomonocytic leukemia, acute monocytic leukemia, acute erythroleukemia, chronic leukemia, chronic myelocytic leukemia, chronic lymphocytic leukemia, polycythemia vera, Hodgkin's disease, non-Hodgkin's disease, Waldenstrom's macroglobulinemia, heavy chain disease, fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilm's tumor, cervical cancer, uterine cancer, testicular cancer, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, craniopharyngioma, pinealoma, hemangioblastoma, acoustic neuroma, schwannoma, melanoma, neuroblastoma, retinoblastoma, lung cancer, squamous cell carcinoma, adenocarcinoma, large cell carcinoma, and colon cancer; or a brain cancer selected from the group consisting of glioblastoma, astrocytoma, glioma, medulloblastoma, oligodendroma, neuroglioma, ependymoma, and meningioma).

In another aspect, the invention features a method of treating (e.g., prophylactically) a subject having heart disease or vascular disease. The method includes administering a composition of the first aspect (e.g., any

composition described herein) to the subject in an amount sufficient to treat the disease (e.g., where the vascular disease is selected from the group consisting of atherosclerosis, restinosis, abdominal aortic aneurysm, thoracic aortic aneurysm, carotid artery disease, peripheral arterial disease, and renal artery disease or where the heart disease is hypertensive heart disease).

In another aspect, the invention features a method of treating a subject having arthritis. The method includes administering a composition of the first aspect (e.g., any composition described herein) to the subject in an amount sufficient to treat the arthritis (e.g., osteoarthritis, rheumatoid arthritis, or any form of arthritis described herein).

In any of the methods described above, the subject may be a human.

By "MT1-MMP inhibitor" is meant a compound (e.g., a soluble polypeptide or polypeptide mimetic) capable of decreasing (e.g., by at least 1%, 5%, 10%, 15%, 25%, 50%, 75%, 85%, 90%, 9%, 98%, 99%, 99.9%) at least one MT1-MMP activity. MT1-MMP activities include activation of proMMP-2 and degradation of proteins including collagen I, collagen II, collagen III, gelatin, fibronectin, Ln-1, vitronectin, aggrecan, tenascin, nidogen, perlecan, fibrinogen/fibrin, fibrillin, α1PI, α2M, Ln-5, CD44, and tTG. Other MT1-MMP activities are described herein and are known in the art.

By an MT1-MMP inhibitor that "selectively inhibits" is meant an inhibitor that is capable of decreasing at least one MT1-MMP activity (e.g., by binding MT1-MMP), but does not substantially reduce activity of (e.g., does not substantially bind to) other proteins (e.g., other membrane metalloproteinases).

By "delivery vector" is meant a moiety or compound, when attached to a therapeutic, that is capable of increasing transport of the therapeutic across a biological barrier (e.g., across a cell membrane or across the blood-brain barrier), as compared to in the absence of the moiety or compound (e.g., a polypeptide).

By a delivery vector that is "efficiently transported across the BBB" is meant a vector that is able to cross the BBB at least as efficiently as Angiopep-

6 (i.e., greater than 38.5% that of Angiopep-1 (250 nM) in the in situ brain perfusion assay described in WO 2008/144919). Accordingly, a vector or conjugate that is "not efficiently transported across the BBB" is transported to the brain at lower levels (e.g., transported less efficiently than Angiopep-6).

By a vector or conjugate which is "efficiently transported to a particular cell type" is meant a vector or conjugate that is able to accumulate (e.g., either due to increased transport into the cell, decreased efflux from the cell, or a combination thereof) in that cell type at least 10% (e.g., 25%, 50%, 100%, 200%, 500%, 1,000%, 5,000%, or 10,000%) greater extent than either a control substance, or, in the case of a conjugate, as compared to the unconjugated agent (e.g., MT1-MMP inhibitor).

By "substantial identity" or "substantially identical" is meant a polypeptide or polynucleotide sequence that has the same polypeptide or polynucleotide sequence, respectively, as a reference sequence, or has a specified percentage of amino acid residues or nucleotides, respectively, that are the same at the corresponding location within a reference sequence when the two sequences are optimally aligned. For example, an amino acid sequence that is "substantially identical" to a reference sequence has at least 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to the reference amino acid sequence. For polypeptides, the length of comparison sequences will generally be at least 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 50, 75, 90, 100, 150, 200, 250, 300, or 350 contiguous amino acids (e.g., a full-length sequence). For nucleic acids, the length of comparison sequences will generally be at least 5, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 contiguous nucleotides (e.g., the full-length nucleotide sequence). Sequence identity may be measured using sequence analysis software on the default setting (e.g., Sequence Analysis Software Package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, 1710 University Avenue, Madison, WI 53705). Such software may match similar sequences by assigning degrees of homology to various substitutions, deletions, and other modifications.

By "substantially pure" or "isolated" is meant a compound (e.g., a polypeptide or conjugate) that has been separated from other chemical components. Typically, the compound is substantially pure when it is at least 30%, by weight, free from other components. In certain embodiments, the preparation is at least 50%, 60%, 75%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% by weight, free from other components. A purified polypeptide may be obtained, for example, by expression of a recombinant polynucleotide encoding such a polypeptide or by chemically synthesizing the polypeptide. Purity can be measured by any appropriate method, for example, column chromatography, polyacrylamide gel electrophoresis, or by HPLC analysis.

By "increased" is meant an increase of at least 5%, 10%, 25%, 25%, 50%, 75%, 100%, 150%, 200%, 500%, or 1000% as compared to a control value.

By "control" is meant a value or amount associated with a healthy or normal subject (e.g., a subject not having a cancer or disease associated with increased MT1-MMP activity).

By "treating" a disease, disorder, or condition in a subject is meant reducing at least one symptom of the disease, disorder, or condition by administrating a therapeutic agent to the subject.

By "treating prophylactically" a disease, disorder, or condition in a subject is meant reducing the frequency of occurrence or severity of a disease, disorder, or condition (e.g., preventing) by administering a therapeutic agent to the subject prior to manifestation of the disease.

By "an effective amount" is meant the amount of a compound, alone or in combination with another therapeutic regimen, required to treat a patient in a clinically relevant manner.

By "subject" is meant a human or non-human animal (e.g., a mammal).

By "vascular disease" is meant any disease associated with changes to the vascular structure. Such diseases include atherosclerosis, restinosis, abdominal aortic aneurysm (AAA), thoracic aortic aneurysm, carotid artery disease, peripheral arterial disease (PAD), and renal artery disease.

Other features and advantages of the invention will be apparent from the following Detailed Description, the drawings, and the claims.

Brief Description of the Drawings

Figure 1 is a schematic diagram showing the structure of MT1-MMP and the sequence of the M14, ACM-14, and scACM-14 polypeptides.

Figure 2 is a set of images showing that expression of nonphosphorylatable MT1-MMP in HT-1080 fibrosarcoma cells reduces tumor growth of those cells when implanted into athymic nude mice.

Figure 3 is a set of photomicrographs showing uptake of scACM-14 and ACM-14 into cells.

Figure 4 is a photograph of a western blot showing inhibition of MT1-MMP phosphorylation by ACM-14, but not scACM-14.

Figure 5 is a set of graphs showing tumor growth in 2D and 3D culture of glioblastoma cells (U-87), medulloblastoma cells (DAOY), prostate adinocarcinoma cells (PC-3), breast adenocarcinoma cells (MDA-MB-231), osteosarcoma cells (MG-63), and fibrosarcoma cells (HT-1080) in the presence or absence of ACM-14 or scACM-14. Three dimensional tumor growth reduction in the presence of ACM-14 was observed in all cells.

Figure 6 is a graph showing tumor size of implanted fibrosarcoma cells in nude, athymic mice with treatment of vehicle alone, scACM-14, or ACM-14. ACM-14 treatment is observed to reduce tumor size significantly.

Figure 7 is a graph showing survival of nude athymic mice with implanted fibrosarcoma cells receiving vehicle alone, scACM-14, or ACM-14. Mice receiving ACM-14 exhibited longer survival times than mice receiving the vehicle or scACM-14.

Figure 8 is a schematic diagram of an Angiopep-ACM-14 conjugate.

Detailed Description

We have discovered that a soluble polypeptide containing a nonphosphorylatable form of the cytoplasmic domain of membrane-type 1

metalloproteinase (MT1-MMP) is capable of inhibiting MT1-MMP activity. As MT1-MMP activity is associated with diseases including cancer, arthritis, fibrosis, and atherosclerosis cells, the MT1-MMP inhibitors described herein can be used in treatment of these diseases.

We have further shown that an MT1-MMP inhibitory polypeptide, when conjugated to a vector capable of delivery the polypeptides to the cytoplasm of cells, is capable of inhibiting cancer growth upon administration to a mammal. Such vectors include the third helix of the homeodomain of the Antennapedia protein, which is capable of penetrating cell membranes. The MT1-MMP polypeptide/antennapedia third helix homeodomain fusion protein inhibits tumor formation and increases survival in mice having cancer tumors, thus providing proof-of-principle that such proteins can be used to treat diseases where decreased MT1-MMP activity is desired, such as cancer, heart and vascular disease, and arthritis.

MT1-MMP and its biological activities

MT1-MMP is a membrane-bound collagenase. Its activities are described, for example, in Itoh et al., J Cell Physiol 206:108, 2006. Increases in MT1-MMP activity are associated with increased degradation of the extracellular matrix and increased cancer invasion, growth, and angiogenesis. MT1-MMP is involved in activation of membrane metalloproteinase-2, which degrades type IV collagen, a component of basement membrane. MT1-MMP is negatively regulated by proteolytic processing, which removes the catalytic domain. This processing results in MT1-MMP downregulation.

Substrates of MT1-MMP include collagen I, collagen II, collagen III, gelatin, fibronectin, Ln-1, vitronectin, aggrecan, tenascin, nidogen, perlecan, fibrinogen/fibrin, fibrillin, α1PI, α2M, Ln-5, CD44, and tTG (see, e.g., Hijova et al., Bratisl Lek Listy 106:127-132, 2005).

MT1-MMP is phosphorylated by Src kinase at Tyr^{573}. MT1-MMP phosphorylation is increased in HT-1080 fibrosarcoma cells when stimulated with the chemoattractant sphingosine-1-phosphate (S1P). S1P increases

endothelial cell micrgration and differentiation into capillary-like structures. Further, subcellular localization of phosphorylated MT1-MMP to the cell periphery is induced when the cells are stimulated with S1P. Finally, phosphorylation of MT1-MMP is important growth in 3D collagen matrices and in anchorage-independent growth of tumor cells (Nyalendo et al., Carcinogenesis 29:1655-1664, 2008).

MT1-MMP and disease

MT1-MMP has been linked to diseases involving degradation of extracellular matrix (ECM) proteins. MT1-MMP activity has, for example, been shown to play important role in tumor cell migration and invasion. MT1-MMP proteolyses ECM proteins (d'Ortho et al., Eur J Biochem 250:751-757, 1997; Hiraoka et al., Cell 95:365-377, 1998; Pei et al., J Biol Chem 271:9135-9140, 1996) as well as a number of cell surface-associated adhesion receptors (Belkin et al., J Biol Chem 276:18415-18422, 2001; Kajita et al., J Cell Biol 153:893-904, 2001). MT1-MMP is overexpressed in many types of tumors (Nakada et al., Am J Pathol 154, 417-428, 1999; Zhai et al., Cancer Res 65, 6543-6550, 2005) and pericellular proteolysis of the dense cross-linked meshwork of type I collagen fibrils mediated by the enzyme confers neoplastic cells with tissue-invasive activity (Sabeh et al., J Cell Biol 167, 769-781, 2004) and sustains tumor cell growth in otherwise growth-restrictive three-dimensional (3D) matrices (Hotary et al., Cell 114, 33-45, 2003).

MT1-MMP expression has also been linked to arthritis, including osteoarthritis and rheumatoid arthritis. Degradation of ECM proteins, such as collagen, in cartilage, is a feature of arthritis. Elevated levels of MT1-MMP, which can act a collagenase, have been observed in both osteoarthritis (Imai et al., Am J Pathol 151:245-256, 1997) and rheumatoid arthritis (Konttinen et al., Ann Rheum Dis 58:691-697, 1999). MT1-MMP activity is thus believed to play a role in arthritic diseases.

MT1-MMP activity has also been linked to vascular disease. MT1-MMP is expressed in smooth muscle cells and in macrophages in

atherosclerotic plaques. MT1-MMP expression is upregulated by proinflammatory molecules in these cells (Tripathi et al., Circulation 99:3103-3109, 1999). As vascular diseases include changes in endothelial cells and smooth muscle structure, MT1-MMP is believed to be involved in the progression of atherosclerosis.

MT1-MMP inhibitors

The compositions of the invention include an MT1-MMP inhibitor, such as a polypeptide or peptidomimetic capable of inhibiting MT1-MMP activity (e.g., any MT1-MMP activity described herein. In certain embodiments, the polypeptide includes an amino acid sequence substantially identical (e.g., 60%, 70%, 80%, 85%, 90%, 95%, 98%) to the cytoplasmic domain of human MT1-MMP (RRHGTPRRLLYCQRSLLDKV; SEQ ID NO:117). The polypeptide may have a substitution, modification, or deletion at the tyrosine at the position corresponding to position 573 of the human MT1-MMP sequence. The tyrosine may be substituted with any naturally occurring amino acid (e.g., Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, and Val), a non-naturally occurring amino acid, or a modified form thereof. In certain embodiments, the tyrosine is substituted with a phenylalanine (e.g., RRHGTPRRLLFCQRSLLDKV; SEQ ID NO:118). In other embodiments, the tyrosine is substituted with a tyrosine analog. Exemplary tyrosine analogs are described in U.S. Patent No. 6,469,047 and in PCT Publication WO 2002/085923 (e.g., Figure 26).

In other embodiments, the MT1-MMP inhibitor polypeptide includes an amino acid sequence substantially identical to a fragment of the MT1-MMP cytoplasmic domain. The fragment may have a substitution, modification, or deletion at the Tyr^{573} of the human MT1-MMP sequence (e.g., those described herein). In certain embodiments, the fragment has 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 amino acids deleted from the N-terminus of the MT1-MMP cytoplasmic domain, 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 amino acids deleted from the C-terminus of the MT1-MMP cytoplasmic

domain, or a combination thereof. In certain embodiments, the fragment contains an amino acid at the position corresponding to residue 573 of the human MT1-MMP sequence.

Any of the MT1-MMP inhibitory polypeptides may have 0, 1, 2, 3, 4, 5, 6, or 7 substitutions (e.g., conservative or nonconservative) as compared to the human wild-type sequence. In certain embodiments, the polypeptide may be a peptidomimetic (e.g., those described herein).

Delivery vectors

The MT1-MMP inhibitory therapeutics described herein may be present in conjunction with a delivery vector capable of enhancing delivery of the therapeutic to target cell(s), or to improve transport of the therapeutic across a biological barrier such as the blood-brain barrier (BBB). Delivery vectors include cell-penetrating peptides, peptides capable of being transported across the BBB, and liposomes capable of enhancing delivery to particular cell types. In certain embodiments, a polypeptide delivery vector forms a fusion protein with the MT1-MMP inhibitor. In other embodiments, the delivery vector is chemically conjugated to the MT1-MMP inhibitor.

Cell penetrating peptide vectors

In certain embodiments, the delivery vector is a cell penetrating peptide. Such peptides are capable, upon couple of the cell penetrating peptide to the therapeutic, of delivering the therapeutic across the cell membrane. In certain embodiments, the cell penetrating peptide is the third helix of the homeodomain of the antennapedia protein (RQIKIWFQNRRMKWKK; SEQ ID NO:119)), or a related peptide (e.g., antennapedia leader peptide (CT) (KKWKMRRNQFWVKVQRG; SEQ ID NO:120), antennapedia peptide amide (RQIKIWFQNRRMKWKK-NH_2; SEQ ID NO:121), and Cys(Npys)-antennapedia peptide amide (C(Npys)-RQIKIWFQNRRMKWKK-NH_2: SEQ ID NO:122).

PATENT
ATTORNEY DOCKET NO. V82819WO

In certain embodiments, the peptide is a cytoplasmic transduction peptide (CTP). Such peptides are described in U.S. Patent No. 7,101,844 and include the amino acid sequences YGRRARRRRRR (SEQ ID NO:123), YGRRARRRARR (SEQ ID NO:124), YGRRARRAARR (SEQ ID NO:125), YKRKARRAARR (SEQ ID NO:126), YARKARRAARR (SEQ ID NO:127), YKRAARRAARR (SEQ ID NO:128), YEREARRAARR (SEQ ID NO:129), YAREARRAARR (SEQ ID NO:130), YGRAARRAARR (SEQ ID NO:131), YRRAARRAARA (SEQ ID NO:132), YPRAARRAARR (SEQ ID NO:133), PARAARRAARR (SEQ ID NO:134), YGRRRRRRRRR (SEQ ID NO:135), and YRRRRRRRRRR (SEQ ID NO:136).

In other embodiments, the peptide is HSV-1 VP22, $(Arg)_9$ (SEQ ID NO:137), Cys(Npys)-$(Arg)_9$ (SEQ ID NO:138), Cys(Npys)-$(D\text{-}Arg)_9$, [Cys^{58}]105Y cell penetrating peptide (CSIPPEVKFNKPFVYLI; SEQ ID NO:139), peptide 105Y (SIPPEVKFNKPFVYLI; SEQ ID NO:140), buforin (TRSSRAGLQFPVGRVHRLLRK; SEQ ID NO:141), chimeric rabies virus glycoprotein fragment (RVG-9R) (YTIWMPENPRPGTPCDIFTNSRGKRASNGGGGRRRRRRRRR; SEQ ID NO:142), Cys(Npys)-TAT(47-57) (C(Npys)YGRKKRRQRRR-NH_2; SEQ ID NO:143), Cys-TAT(47-57) (CYGRKKRRQRRR-NH_2; SEQ ID NO:144), lipid membrane translocating peptide (KKAAAVLLPVLLAAP; SEQ ID NO:145), D-isomer-lipid membrane translocating peptide ("All D" KKAAAVLLPVLLAAP), mastoparan (INLKALAALAKKIL-NH_2; SEQ ID NO:146), mastoparan 7 (INLKALAALAKALL-NH_2; SEQ ID NO:147), mastoparan X (INWKGIAAMAKKLL-NH_2; SEQ ID NO:148), MEK1 derived peptide inhibitor 1 (MPKKKPTPIQLNP; SEQ ID NO:149), myristoyl-MEK1 derived peptide inhibitor 1 (Myr-MPKKKPTPIQLNP; SEQ ID NO:150), stearyl-MEK-1 derived peptide inhibitor 1 amide (Ste-MPKKKPTPIQLNP-NH_2; SEQ ID NO:151), membrane-permeable sequence (MPS; AAVALLPAVLLALLAK; SEQ ID NO:152), HIV related MPG (GALFLGFLGAAGSTMGAWSQPKSKRKV; SEQ ID NO:153), aminopeptidase N ligand (CD13), NGR peptide (CNGRCG, Cys1-Cys5

disulfide bridge; SEQ ID NO:154), NGR peptide 1 (Cys-Asn-Gly-Arg-Cys-Gly-Gly-D-Lys-D-Leu-D-Ala-D-Lys-D-Leu-D-Ala-D-Lys-D-Lys-D-Leu-D-Ala-D-Lys-D-Leu-D-Ala-D-Lys-NH_2 (Disulfide bridge: 1-5)), NGR peptide 2 (CNGRCGGLVTT (disulfide bridge: 1-5); SEQ ID NO:155), NGR peptide 3 (CNGRC-NH_2 (Disulfide bridge: 1-5); SEQ ID NO:156), NGR peptide 4 (Cys-Asn-Gly-Arg-Cys-Gly-Gly-D-Lys-D-Lys-D-Leu-D-Lys-D-Leu-D-Leu-D-Leu-D-Lys-D-Leu-D-Leu-OH (Disulfide bridge: 1-5)), Pep-1 (Chariot™; KETWWETWWTEWSQPKKKRKV; SEQ ID NO:157), SynB1 (RGGRLSYSRRRFSTSTGRA; SEQ ID NO:158), biotin-TAT(47-57) (biotin-YGRKKRRQRRR; SEQ ID NO:159), TAT(47-57) (YGRKKRRQRRR; SEQ ID NO:160), TAT(47-57) GGG-Cys(Npys) (YGRKKRRQRRRGGG-C(Npys)-NH_2; SEQ ID NO:161), TAT(48-57) (GRKKRRQRRR; SEQ ID NO:162), Tat-C(48-57) (CGRKKRRQRRR; SEQ ID NO:163), transdermal peptide (ACSSSPSKHCG; SEQ ID NO:164), transportan (GWTLNSAGYLLGKINLKALAALAKKIL; SEQ ID NO:165), and transportan 10 (AGYLLGKINLKALAALAKKIL-NH_2; SEQ ID NO:166).

Polypeptide vectors capable of entering or accumulating in cells

We have developed polypeptides capable of entering and accumulating in cells types such as liver, lungs, kidneys, spleen, and muscle. See, e.g., PCT Publication WO 2008/144919. Such peptides include Angiopep-7 (TFFYGGSRGRRNNFRTEEY; SEQ ID NO:112), which is capable of entering these cell types, but does not efficiently cross the blood-brain barrier. Other peptides capable of entering and accumulating in these cell types include the Angiopep peptides described below, which also cross the BBB.

In certain embodiments, the polypeptide includes an amino acid sequence substantially identical to Angiopep-7. The polypeptide may include a sequence having 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acid substitutions as compared to the Angiopep-7 sequence, or may be a fragment of Angiopep-7 (e.g., a fragment capable of accumulating in at least cell type selected from the group consisting of liver, lung, spleen, muscle, or pancreas). In certain

embodiments, the Angiopep-7 polypeptide may include either an N-terminal or a C-terminal cysteine residue (e.g., CTFFYGGSRGRRNNFRTEEY (SEQ ID NO:115) and TFFYGGSRGRRNNFRTEEYC (SEQ ID NO:116)).

In certain embodiments, the polypeptide includes an amino acid sequence having the formula:

X1-X2-X3-X4-X5-X6-X7-X8-X9-X10-X11-X12-X13-X14-X15-X16-X17-X18-X19

where each of X1-X19 (e.g., X1-X6, X8, X9, X11-X14, and X16-X19) is, independently, any amino acid (e.g., a naturally occurring amino acid such as Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, and Val) or absent and at least one (e.g., 2 or 3) of X1, X10, and X15 is arginine. In some embodiments, X7 is Ser or Cys; or X10 and X15 each are independently Arg or Lys. In some embodiments, the residues from X1 through X19, inclusive, are substantially identical to any of the amino acid sequences of any one of SEQ ID NOS:1-105 and 107-116 (e.g., Angiopep-1, Angiopep-2, Angiopep-3, Angiopep-4a, Angiopep-4b, Angiopep-5, Angiopep-6, and Angiopep-7). In some embodiments, at least one (e.g., 2, 3, 4, or 5) of the amino acids X1-X19 is Arg. In some embodiments, the polypeptide has one or more additional cysteine residues at the N-terminal of the polypeptide, the C-terminal of the polypeptide, or both. The polypeptide may be capable of accumulating in particular cell types, and may (or may not) efficiently cross the BBB.

Delivery vectors capable of crossing the blood-brain barrier

In certain embodiments, the therapeutic is attached to a delivery vector capable of crossing the blood-brain barrier. Such polypeptides include the aprotinin-derived Angiopep series of peptides and analogs thereof, including aprotinin (e.g., as described in U.S. Patent Application No. 2006/0189515), antibodies, and others described herein.

PATENT
ATTORNEY DOCKET NO. V82819WO

Angiopep peptides

In certain embodiments, the therapeutic is attached to a polypeptide substantially identical to any of the sequences set Table 1, or a fragment thereof. Particular examples of such polypeptides are those having the sequence of Angiopep-1 (SEQ ID NO:67), Angiopep-2 (SEQ ID NO:97), Angiopep-3 (SEQ ID NO:107), Angiopep-4a (SEQ ID NO:108), Angiopep-4b (SEQ ID NO:109), Angiopep-5 (SEQ ID NO:110), or Angiopep-6 (SEQ ID NO:111). The peptide vector may be of any length, for example, at least 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 25, 35, 50, 75, 100, 200, or 500 amino acids, or any range between these numbers. In certain embodiments, the peptide vector is 10 to 50 amino acids in length. The polypeptide may be produced by recombinant genetic technology or chemical synthesis. The polypeptide may contain a C-terminal cysteine, an N-terminal cysteine, or both.

Table 1: Exemplary Polypeptides

SEQ ID NO:																		
1	T	F	V	Y	G	G	C	R	A	K	R	N	N	F	K	S	A	E	D
2	T	F	Q	Y	G	G	C	M	G	N	G	N	N	F	V	T	E	K	E
3	P	F	F	Y	G	G	C	G	G	N	R	N	N	F	D	T	E	E	Y
4	S	F	Y	Y	G	G	C	L	G	N	K	N	N	Y	L	R	E	E	E
5	T	F	F	Y	G	G	C	R	A	K	R	N	N	F	K	R	A	K	Y
6	T	F	F	Y	G	G	C	R	G	K	R	N	N	F	K	R	A	K	Y
7	T	F	F	Y	G	G	C	R	A	K	K	N	N	Y	K	R	A	K	Y
8	T	F	F	Y	G	G	C	R	G	K	K	N	N	F	K	R	A	K	Y
9	T	F	Q	Y	G	G	C	R	A	K	R	N	N	F	K	R	A	K	Y
10	T	F	Q	Y	G	G	C	R	G	K	K	N	N	F	K	R	A	K	Y
11	T	F	F	Y	G	G	C	L	G	K	R	N	N	F	K	R	A	K	Y
12	T	F	F	Y	G	G	S	L	G	K	R	N	N	F	K	R	A	K	Y
13	P	F	F	Y	G	G	C	G	G	K	K	N	N	F	K	R	A	K	Y
14	T	F	F	Y	G	G	C	R	G	K	G	N	N	Y	K	R	A	K	Y
15	P	F	F	Y	G	G	C	R	G	K	R	N	N	F	L	R	A	K	Y
16	T	F	F	Y	G	G	C	R	G	K	R	N	N	F	K	R	E	K	Y
17	P	F	F	Y	G	G	C	R	A	K	K	N	N	F	K	R	A	K	E
18	T	F	F	Y	G	G	C	R	G	K	R	N	N	F	K	R	A	K	D
19	T	F	F	Y	G	G	C	R	A	K	R	N	N	F	D	R	A	K	Y
20	T	F	F	Y	G	G	C	R	G	K	K	N	N	F	K	R	A	E	Y
21	P	F	F	Y	G	G	C	G	A	N	R	N	N	F	K	R	A	K	Y

PATENT
ATTORNEY DOCKET NO. V82819WO

22	T	F	F	Y	G	G	C	G	G	K	K	N	N	F	K	T	A	K	Y	
23	T	F	F	Y	G	G	C	R	G	N	R	N	N	F	L	R	A	K	Y	
24	T	F	F	Y	G	G	C	R	G	N	R	N	N	F	K	T	A	K	Y	
25	T	F	F	Y	G	G	S	R	G	N	R	N	N	F	K	T	A	K	Y	
26	T	F	F	Y	G	G	C	L	G	N	G	N	N	F	K	R	A	K	Y	
27	T	F	F	Y	G	G	C	L	G	N	R	N	N	F	L	R	A	K	Y	
28	T	F	F	Y	G	G	C	L	G	N	R	N	N	F	K	T	A	K	Y	
29	T	F	F	Y	G	G	C	R	G	N	G	N	N	F	K	S	A	K	Y	
30	T	F	F	Y	G	G	C	R	G	K	K	N	N	F	D	R	E	K	Y	
31	T	F	F	Y	G	G	C	R	G	K	R	N	N	F	L	R	E	K	E	
32	T	F	F	Y	G	G	C	R	G	K	G	N	N	F	D	R	A	K	Y	
33	T	F	F	Y	G	G	S	R	G	K	G	N	N	F	D	R	A	K	Y	
34	T	F	F	Y	G	G	C	R	G	N	G	N	N	F	V	T	A	K	Y	
35	P	F	F	Y	G	G	C	G	G	K	G	N	N	Y	V	T	A	K	Y	
36	T	F	F	Y	G	G	C	L	G	K	G	N	N	F	L	T	A	K	Y	
37	S	F	F	Y	G	G	C	L	G	N	K	N	N	F	L	T	A	K	Y	
38	T	F	F	Y	G	G	C	G	G	N	K	N	N	F	V	R	E	K	Y	
39	T	F	F	Y	G	G	C	M	G	N	K	N	N	F	V	R	E	K	Y	
40	T	F	F	Y	G	G	S	M	G	N	K	N	N	F	V	R	E	K	Y	
41	P	F	F	Y	G	G	C	L	G	N	R	N	N	Y	V	R	E	K	Y	
42	T	F	F	Y	G	G	C	L	G	N	R	N	N	F	V	R	E	K	Y	
43	T	F	F	Y	G	G	C	L	G	N	K	N	N	Y	V	R	E	K	Y	
44	T	F	F	Y	G	G	C	G	G	N	G	N	N	F	L	T	A	K	Y	
45	T	F	F	Y	G	G	C	R	G	N	R	N	N	F	L	T	A	E	Y	
46	T	F	F	Y	G	G	C	R	G	N	G	N	N	F	K	S	A	E	Y	
47	P	F	F	Y	G	G	C	L	G	N	K	N	N	F	K	T	A	E	Y	
48	T	F	F	Y	G	G	C	R	G	N	R	N	N	F	K	T	E	E	Y	
49	T	F	F	Y	G	G	C	R	G	K	R	N	N	F	K	T	E	E	D	
50	P	F	F	Y	G	G	C	G	G	N	G	N	N	F	V	R	E	K	Y	
51	S	F	F	Y	G	G	C	M	G	N	G	N	N	F	V	R	E	K	Y	
52	P	F	F	Y	G	G	C	G	G	N	G	N	N	F	L	R	E	K	Y	
53	T	F	F	Y	G	G	C	L	G	N	G	N	N	F	V	R	E	K	Y	
54	S	F	F	Y	G	G	C	L	G	N	G	N	N	Y	L	R	E	K	Y	
55	T	F	F	Y	G	G	S	L	G	N	G	N	N	F	V	R	E	K	Y	
56	T	F	F	Y	G	G	C	R	G	N	G	N	N	F	V	T	A	E	Y	
57	T	F	F	Y	G	G	C	L	G	K	G	N	N	F	V	S	A	E	Y	
58	T	F	F	Y	G	G	C	L	G	N	R	N	N	F	D	R	A	E	Y	
59	T	F	F	Y	G	G	C	L	G	N	R	N	N	F	L	R	E	E	Y	
60	T	F	F	Y	G	G	C	L	G	N	K	N	N	Y	L	R	E	E	Y	
61	P	F	F	Y	G	G	C	G	G	N	R	N	N	Y	L	R	E	E	Y	
62	P	F	F	Y	G	G	S	G	G	N	R	N	N	Y	L	R	E	E	Y	
63	M	R	P	D	F	C	L	E	P	P	Y	T	G	P	C	V	A	R	I	
64	A	R	I	I	R	Y	F	Y	N	A	K	A	G	L	C	Q	T	F	V	Y	G	
65	Y	G	G	C	R	A	K	R	N	N	Y	K	S	A	E	D	C	M	R	T	C	G

PATENT
ATTORNEY DOCKET NO. V82819WO

66 P D F C L E P P Y T G P C V A R I I R Y F Y
67 T F F Y G G C R G K R N N F K T E E Y
68 K F F Y G G C R G K R N N F K T E E Y
69 T F Y Y G G C R G K R N N Y K T E E Y
70 T F F Y G G S R G K R N N F K T E E Y
71 C T F F Y G C C R G K R N N F K T E E Y
72 T F F Y G G C R G K R N N F K T E E Y C
73 C T F F Y G S C R G K R N N F K T E E Y
74 T F F Y G G S R G K R N N F K T E E Y C
75 P F F Y G G C R G K R N N F K T E E Y
76 T F F Y G G C R G K R N N F K T K E Y
77 T F F Y G G K R G K R N N F K T E E Y
78 T F F Y G G C R G K R N N F K T K R Y
79 T F F Y G G K R G K R N N F K T A E Y
80 T F F Y G G K R G K R N N F K T A G Y
81 T F F Y G G K R G K R N N F K R E K Y
82 T F F Y G G K R G K R N N F K R A K Y
83 T F F Y G G C L G N R N N F K T E E Y
84 T F F Y G C G R G K R N N F K T E E Y
85 T F F Y G G R C G K R N N F K T E E Y
86 T F F Y G G C L G N G N N F D T E E E
87 T F Q Y G G C R G K R N N F K T E E Y
88 Y N K E F G T F N T K G C E R G Y R F
89 R F K Y G G C L G N M N N F E T L E E
90 R F K Y G G C L G N K N N F L R L K Y
91 R F K Y G G C L G N K N N Y L R L K Y
92 K T K R K R K K Q R V K I A Y E E I F K N Y
93 K T K R K R K K Q R V K I A Y
94 R G G R L S Y S R R F S T S T G R
95 R R L S Y S R R R F
96 R Q I K I W F Q N R R M K W K K
97 T F F Y G G S R G K R N N F K T E E Y
98 M R P D F C L E P P Y T G P C V A R I
I R Y F Y N A K A G L C Q T F V Y G G
C R A K R N N F K S A E D C M R T C G G A

99 T F F Y G G C R G K R N N F K T K E Y
100 R F K Y G G C L G N K N N Y L R L K Y
101 T F F Y G G C R A K R N N F K R A K Y
102 N A K A G L C Q T F V Y G G C L A K R N N F
E S A E D C M R T C G G A

103 Y G G C R A K R N N F K S A E D C M R T C G

PATENT
ATTORNEY DOCKET NO. V82819WO

	G	A																			
104	G	L	C	Q	T	F	V	Y	G	G	C	R	A	K	R	N	N	F	K	S	A	E
105	L	C	Q	T	F	V	Y	G	G	C	E	A	K	R	N	N	F	K	S	A		
107	T	F	F	Y	G	G	S	R	G	K	R	N	N	F	K	T	E	E	Y			
108	R	F	F	Y	G	G	S	R	G	K	R	N	N	F	K	T	E	E	Y			
109	R	F	F	Y	G	G	S	R	G	K	R	N	N	F	K	T	E	E	Y			
110	R	F	F	Y	G	G	S	R	G	K	R	N	N	F	R	T	E	E	Y			
111	T	F	F	Y	G	G	S	R	G	K	R	N	N	F	R	T	E	E	Y			
113	C	T	F	F	Y	G	G	S	R	G	K	R	N	N	F	K	T	E	E	Y		
114	T	F	F	Y	G	G	S	R	G	K	R	N	N	F	K	T	E	E	Y	C		

Polypeptides Nos. 5, 67, 76, and 91, include the sequences of SEQ ID NOS:5, 67, 76, and 91, respectively, and are amidated at the C-terminus.
Polypeptides Nos. 107, 109, and 110 include the sequences of SEQ ID NOS:97, 109, and 110, respectively, and are acetylated at the N-terminus.

In certain embodiments, the Angiopep polypeptide is capable of efficiently crossing the BBB and includes an amino acid sequence having the formula:

X1-X2-X3-X4-X5-X6-X7-X8-X9-X10-X11-X12-X13-X14-X15-X16-X17-X18-X19

where each of X1-X19 (e.g., X1-X6, X8, X9, X11-X14, and X16-X19) is, independently, any amino acid (e.g., a naturally occurring amino acid such as Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, and Val) or absent and at least one (e.g., 2 or 3) of X1, X10, and X15 is arginine. In some embodiments, X7 is Ser or Cys; or X10 and X15 each are independently Arg or Lys. In some embodiments, the residues from X1 through X19, inclusive, are substantially identical to any of the amino acid sequences of any one of SEQ ID NOS:1-105 and 107-116 (e.g., Angiopep-1, Angiopep-2, Angiopep-3, Angiopep-4a, Angiopep-4b, Angiopep-5, Angiopep-6, and Angiopep-7). In some embodiments, at least one (e.g., 2, 3, 4, or 5) of the amino acids X1-X19 is Arg. In some embodiments, the polypeptide has one or more additional cysteine residues at the N-terminal of the polypeptide, the C-terminal of the polypeptide, or both.

Antibodies

In certain embodiments, an antibody or antibody fragment that binds a receptor capable of mediating transcytosis across the BBB is used as a delivery vector to transport the MT1-MMP inhibitor across the BBB. Following binding to the receptor, the antibody can be transported across the BBB. Accordingly, such antibodies can be used to transport agents across the BBB, when the agent is conjugated to, or otherwise associated with, such an antibody. These antibodies include anti-insulin receptor antibodies (described in U.S. Patent Application Publication No. 2004/0101904), anti-transferrin receptor antibodies (described in Pardridge et al., Pharmacol. Exp. Ther. 256:66-70, 1991).

Additional antibodies targeted to receptors capable of mediating transcytosis can be generated using methods well known in the art. In certain embodiments, the antibodies are human or are humanized.

Other peptides

Other peptides capable of crossing the BBB that can be used as delivery vectors include rabies virus glycoprotein (RVG; YTIWMPENPRPGTPCDIFTNSRGKRASNG; SEQ ID NO:167), p97, transferrin, insulin, receptor-associated protein (RAP), tissue-type plasminogen activator (tPA), and lactoferrin. LRP ligands, insulin-like growth factor (IGF), leptin, low density lipoprotein (LDL), which may be capable of crossing the BBB, are also described in U.S. Patent Application Publication Nos. 2003/0129186 and 2004/0102369. Still other peptides, such as the H.8 region of the Neisseria gonorrhoeae F62 Laz protein (CSQEPAAPAAEATPAGEAPASEAPAAEAAPADAAEAPAA; SEQ ID NO:168) or peptides having at least 4 perfect or imperfect AAEAP repeats are described U.S. Patent Application Publication No. 2008/0213185. Such peptides are capable of crossing the BBB.

Still other polypeptides include Kunitz domain-containing polypeptides, such as aprotinin, bikunin, amyloid beta precursor protein, and Kunitz inhibitor proteins.

Liposomal delivery systems

The MT1-MMP inhibitor can also be delivered to particular tissues using liposomes. In certain embodiments, the MT1-MMP inhibitory therapeutic (e.g., a polypeptide) is delivered to target cells using an immunoliposomes. In this approach, the polypeptide is encapsulated in a liposome (e.g., any suitable liposome known in the art), where the liposome is conjugated on its exterior surface to an antibody capable of binding to a antigen expressed on the target cell. In one example, the immunoliposome targeted to a cancer cell. Such immunoliposomes can use anti-EGF receptor antibodies (e.g., as described in Mamot et al., Cancer Res 63:3154-61, 2003), anti-HER2 antibodies (e.g., as described in Kirpotin et al., Biochemistry, 36:66–75, 1997 and in Park et al., Proc Natl Acad Sci USA 92:1327-1331, 1995), anti-MUC1 antibodies (e.g., as described in Moase et al., Biochim Biophys Acta 1510:43-55, 2001), anti-CC52 antibodies (Kamps et al., J Drug Target 8:235-45, 2000), antiganglioside G(M3) antibodies (DH2) or anti-Le(x) antibodies (SH1) (e.g., as described in Nam et al., Oncol Res 11:9-16, 1999).

Delivery across the BBB can also be accomplished similarly. For example, a liposome containing an MT1-MMP inhibitor can be conjugated to an antibody directed to a receptor capable of mediating transcytosis across the BBB. Such antibodies are described above and include anti-transferrin receptor antibodies and anti-insulin receptor antibodies.

Polypeptide derivatives and peptidomimetics

In addition to polypeptides consisting of naturally occurring amino acids, peptidomimetics or polypeptide analogs are also encompassed by the present invention and can form the delivery vectors or MT1-MMP inhibitors used in the compositions of the invention. Polypeptide analogs are commonly

used in the pharmaceutical industry as non-peptide drugs with properties analogous to those of the template polypeptide. The non-peptide compounds are termed "peptide mimetics" or peptidomimetics (Fauchere et al., Infect. Immun. 54:283-287,1986 and Evans et al., J. Med. Chem. 30:1229-1239, 1987). Peptide mimetics that are structurally related to therapeutically useful peptides or polypeptides may be used to produce an equivalent or enhanced therapeutic or prophylactic effect. Generally, peptidomimetics are structurally similar to the paradigm polypeptide (i.e., a polypeptide that has a biological or pharmacological activity) such as naturally-occurring receptor-binding polypeptides, but have one or more peptide linkages optionally replaced by linkages such as $-CH_2NH-$, $-CH_2S-$, $-CH_2-CH_2-$, $-CH=CH-$ (cis and trans), $-CH_2SO-$, $-CH(OH)CH_2-$, $-COCH_2-$ etc., by methods well known in the art (Spatola, Peptide Backbone Modifications, Vega Data, 1:267, 1983; Spatola et al., Life Sci. 38:1243-1249, 1986; Hudson et al., Int. J. Pept. Res. 14:177-185, 1979; and Weinstein, 1983, Chemistry and Biochemistry, of Amino Acids, Peptides and Proteins, Weinstein eds, Marcel Dekker, New York). Such polypeptide mimetics may have significant advantages over naturally occurring polypeptides including more economical production, greater chemical stability, enhanced pharmacological properties (e.g., half-life, absorption, potency, efficiency), reduced antigenicity, and others.

While the peptide vectors described herein may efficiently cross the BBB or target particular cell types (e.g., those described herein), their effectiveness may be reduced by the presence of proteases. Likewise, the effectiveness of MT1-MMP inhibitors used in the invention may be similarly reduced. Serum proteases have specific substrate requirements, including L-amino acids and peptide bonds for cleavage. Furthermore, exopeptidases, which represent the most prominent component of the protease activity in serum, usually act on the first peptide bond of the polypeptide and require a free N-terminus (Powell et al., Pharm. Res. 10:1268-1273, 1993). In light of this, it is often advantageous to use modified versions of polypeptides. The modified polypeptides retain the structural characteristics of the original L-

amino acid polypeptides, but advantageously are not readily susceptible to cleavage by protease and/or exopeptidases.

Systematic substitution of one or more amino acids of a consensus sequence with D-amino acid of the same type (e.g., an enantiomer; D-lysine in place of L-lysine) may be used to generate more stable polypeptides. Thus, a polypeptide derivative or peptidomimetic as described herein may be all L-, all D-, or mixed D, L polypeptides. The presence of an N-terminal or C-terminal D-amino acid increases the in vivo stability of a polypeptide because peptidases cannot utilize a D-amino acid as a substrate (Powell et al., Pharm. Res. 10:1268-1273, 1993). Reverse-D polypeptides are polypeptides containing D-amino acids, arranged in a reverse sequence relative to a polypeptide containing L-amino acids. Thus, the C-terminal residue of an L-amino acid polypeptide becomes N-terminal for the D-amino acid polypeptide, and so forth. Reverse D-polypeptides retain the same tertiary conformation and therefore the same activity, as the L-amino acid polypeptides, but are more stable to enzymatic degradation in vitro and in vivo, and thus have greater therapeutic efficacy than the original polypeptide (Brady and Dodson, Nature 368:692-693, 1994; Jameson et al., Nature 368:744-746, 1994). In addition to reverse-D-polypeptides, constrained polypeptides comprising a consensus sequence or a substantially identical consensus sequence variation may be generated by methods well known in the art (Rizo et al., Ann. Rev. Biochem. 61:387-418, 1992). For example, constrained polypeptides may be generated by adding cysteine residues capable of forming disulfide bridges and, thereby, resulting in a cyclic polypeptide. Cyclic polypeptides have no free N- or C-termini. Accordingly, they are not susceptible to proteolysis by exopeptidases, although they are, of course, susceptible to endopeptidases, which do not cleave at polypeptide termini. The amino acid sequences of the polypeptides with N-terminal or C-terminal D-amino acids and of the cyclic polypeptides are usually identical to the sequences of the polypeptides to which they correspond, except for the presence of N-terminal or C-terminal D-amino acid residue, or their circular structure, respectively.

PATENT
ATTORNEY DOCKET NO. V82819WO

A cyclic derivative containing an intramolecular disulfide bond may be prepared by conventional solid phase synthesis while incorporating suitable S-protected cysteine or homocysteine residues at the positions selected for cyclization such as the amino and carboxy termini (Sah et al., J. Pharm. Pharmacol. 48:197, 1996). Following completion of the chain assembly, cyclization can be performed either (1) by selective removal of the S-protecting group with a consequent on-support oxidation of the corresponding two free SH-functions, to form a S-S bonds, followed by conventional removal of the product from the support and appropriate purification procedure or (2) by removal of the polypeptide from the support along with complete side chain de-protection, followed by oxidation of the free SH-functions in highly dilute aqueous solution.

The cyclic derivative containing an intramolecular amide bond may be prepared by conventional solid phase synthesis while incorporating suitable amino and carboxyl side chain protected amino acid derivatives, at the position selected for cyclization. The cyclic derivatives containing intramolecular -S-alkyl bonds can be prepared by conventional solid phase chemistry while incorporating an amino acid residue with a suitable amino-protected side chain, and a suitable S-protected cysteine or homocysteine residue at the position selected for cyclization.

Another effective approach to confer resistance to peptidases acting on the N-terminal or C-terminal residues of a polypeptide is to add chemical groups at the polypeptide termini, such that the modified polypeptide is no longer a substrate for the peptidase. One such chemical modification is glycosylation of the polypeptides at either or both termini. Certain chemical modifications, in particular N-terminal glycosylation, have been shown to increase the stability of polypeptides in human serum (Powell et al., Pharm. Res. 10:1268-1273, 1993). Other chemical modifications which enhance serum stability include, but are not limited to, the addition of an N-terminal alkyl group, consisting of a lower alkyl of from one to twenty carbons, such as an acetyl group, and/or the addition of a C-terminal amide or substituted amide

group. In particular, the present invention includes modified polypeptides consisting of polypeptides bearing an N-terminal acetyl group and/or a C-terminal amide group.

Also included by the present invention are other types of polypeptide derivatives containing additional chemical moieties not normally part of the polypeptide, provided that the derivative retains the desired functional activity of the polypeptide. Examples of such derivatives include (1) N-acyl derivatives of the amino terminal or of another free amino group, wherein the acyl group may be an alkanoyl group (e.g., acetyl, hexanoyl, octanoyl) an aroyl group (e.g., benzoyl) or a blocking group such as F-moc (fluorenylmethyl-O–CO–); (2) esters of the carboxy terminal or of another free carboxy or hydroxyl group; (3) amide of the carboxy-terminal or of another free carboxyl group produced by reaction with ammonia or with a suitable amine; (4) phosphorylated derivatives; (5) derivatives conjugated to an antibody or other biological ligand and other types of derivatives.

Longer polypeptide sequences which result from the addition of additional amino acid residues to the polypeptides described herein are also encompassed in the present invention. Such longer polypeptide sequences can be expected to have the same biological activity and specificity (e.g., cell tropism) as the polypeptides described above. While polypeptides having a substantial number of additional amino acids are not excluded, it is recognized that some large polypeptides may assume a configuration that masks the effective sequence, thereby preventing binding to a target. These derivatives could act as competitive antagonists. Thus, while the present invention encompasses polypeptides or derivatives of the polypeptides described herein having an extension, desirably the extension does not destroy the cell targeting activity of the polypeptides or its derivatives.

Other derivatives included in the present invention are dual polypeptides consisting of two of the same, or two different polypeptides, as described herein, covalently linked to one another either directly or through a spacer, such as by a short stretch of alanine residues or by a putative site for

proteolysis (e.g., by cathepsin, see e.g., U.S. Patent No. 5,126,249 and European Patent No. 495 049). Multimers of the polypeptides described herein consist of a polymer of molecules formed from the same or different polypeptides or derivatives thereof.

The present invention also encompasses polypeptide derivatives that are chimeric or fusion proteins containing a polypeptide described herein, or fragment thereof, linked at its amino- or carboxy-terminal end, or both, to an amino acid sequence of a different protein. Such a chimeric or fusion protein may be produced by recombinant expression of a nucleic acid encoding the protein. For example, a chimeric or fusion protein may contain at least 6 amino acids shared with one of the described polypeptides which desirably results in a chimeric or fusion protein that has an equivalent or greater functional activity.

Assays to identify peptidomimetics

As described above, non-peptidyl compounds generated to replicate the backbone geometry and pharmacophore display (peptidomimetics) of the polypeptides described herein often possess attributes of greater metabolic stability, higher potency, longer duration of action, and better bioavailability.

Peptidomimetics compounds can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including biological libraries, spatially addressable parallel solid phase or solution phase libraries, synthetic library methods requiring deconvolution, the 'one-bead one-compound' library method, and synthetic library methods using affinity chromatography selection. The biological library approach is limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer, or small molecule libraries of compounds (Lam, Anticancer Drug Des. 12:145, 1997). Examples of methods for the synthesis of molecular libraries can be found in the art, for example, in: DeWitt et al. (Proc. Natl. Acad. Sci. USA 90:6909, 1993); Erb et al. (Proc. Natl. Acad. Sci. USA 91:11422, 1994); Zuckermann et al. (J. Med. Chem. 37:2678, 1994); Cho et al. (Science 261:1303, 1993); Carell et al. (Angew. Chem, Int. Ed. Engl. 33:2059,

1994 and ibid 2061); and in Gallop et al. (Med. Chem. 37:1233, 1994). Libraries of compounds may be presented in solution (e.g., Houghten, Biotechniques 13:412-421, 1992) or on beads (Lam, Nature 354:82-84, 1991), chips (Fodor, Nature 364:555-556, 1993), bacteria or spores (U.S. Patent No. 5,223,409), plasmids (Cull et al., Proc. Natl. Acad. Sci. USA 89:1865-1869, 1992) or on phage (Scott and Smith, Science 249:386-390, 1990), or luciferase, and the enzymatic label detected by determination of conversion of an appropriate substrate to product.

Once a polypeptide as described herein is identified, it can be isolated and purified by any number of standard methods including, but not limited to, differential solubility (e.g., precipitation), centrifugation, chromatography (e.g., affinity, ion exchange, and size exclusion), or by any other standard techniques used for the purification of peptides, peptidomimetics, or proteins. The functional properties of an identified polypeptide of interest may be evaluated using any functional assay known in the art. Desirably, assays for evaluating downstream receptor function in intracellular signaling are used (e.g., cell proliferation).

For example, the peptidomimetics compounds of the present invention may be obtained using the following three-phase process: (1) scanning the polypeptides described herein to identify regions of secondary structure necessary for targeting the particular cell types described herein; (2) using conformationally constrained dipeptide surrogates to refine the backbone geometry and provide organic platforms corresponding to these surrogates; and (3) using the best organic platforms to display organic pharmocophores in libraries of candidates designed to mimic the desired activity of the native polypeptide. In more detail the three phases are as follows. In phase 1, the lead candidate polypeptides are scanned and their structure abridged to identify the requirements for their activity. A series of polypeptide analogs of the original are synthesized. In phase 2, the best polypeptide analogs are investigated using the conformationally constrained dipeptide surrogates. Indolizidin-2-one, indolizidin-9-one and quinolizidinone amino acids (I^2aa, I^9aa

and Qaa respectively) are used as platforms for studying backbone geometry of the best peptide candidates. These and related platforms (reviewed in Halab et al., Biopolymers 55:101-122, 2000 and Hanessian et al., Tetrahedron 53:12789-12854, 1997) may be introduced at specific regions of the polypeptide to orient the pharmacophores in different directions. Biological evaluation of these analogs identifies improved lead polypeptides that mimic the geometric requirements for activity. In phase 3, the platforms from the most active lead polypeptides are used to display organic surrogates of the pharmacophores responsible for activity of the native peptide. The pharmacophores and scaffolds are combined in a parallel synthesis format. Derivation of polypeptides and the above phases can be accomplished by other means using methods known in the art.

Structure function relationships determined from the polypeptides, polypeptide derivatives, peptidomimetics or other small molecules described herein may be used to refine and prepare analogous molecular structures having similar or better properties. Accordingly, the compounds of the present invention also include molecules that share the structure, polarity, charge characteristics and side chain properties of the polypeptides described herein.

In summary, based on the disclosure herein, those skilled in the art can develop peptides and peptidomimetics screening assays which are useful for identifying compounds for targeting an agent to particular cell types (e.g., those described herein). The assays of this invention may be developed for low-throughput, high-throughput, or ultra-high throughput screening formats. Assays of the present invention include assays amenable to automation.

Linkers

In embodiments where the MT1-MMP is covalently bound to a delivery vector, the MT1-MMP inhibitor can be bound to a delivery vector either directly (e.g., through a covalent bond such as a peptide bond) or can be bound through a linker. Linkers include chemical linking agents (e.g., cleavable linkers) and peptides.

In some embodiments, the linker is a chemical linking agent. The MT1-MMP inhibitor (e.g. a polypeptide or peptidomimetic) and vector peptide may be conjugated through sulfhydryl groups, amino groups (amines), and/or carbohydrates or any appropriate reactive group. Homobifunctional and heterobifunctional cross-linkers (conjugation agents) are available from many commercial sources. Regions available for cross-linking may be found on the polypeptides of the present invention. The cross-linker may comprise a flexible arm, e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 carbon atoms. Exemplary cross-linkers include BS3 ([Bis(sulfosuccinimidyl)suberate]; BS3 is a homobifunctional N-hydroxysuccinimide ester that targets accessible primary amines), NHS/EDC (N-hydroxysuccinimide and 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide; NHS/EDC allows for the conjugation of primary amine groups with carboxyl groups), sulfo-EMCS ([N-e-maleimidocaproic acid]hydrazide; sulfo-EMCS are heterobifunctional reactive groups (maleimide and NHS-ester) that are reactive toward sulfhydryl and amino groups), hydrazide (most proteins contain exposed carbohydrates and hydrazide is a useful reagent for linking carboxyl groups to primary amines), and SATA (N-succinimidyl-S-acetylthioacetate; SATA is reactive towards amines and adds protected sulfhydryls groups).

To form covalent bonds, one can use as a chemically reactive group a wide variety of active carboxyl groups (e.g., esters) where the hydroxyl moiety is physiologically acceptable at the levels required to modify the peptide. Particular agents include N-hydroxysuccinimide (NHS), N-hydroxy-sulfosuccinimide (sulfo-NHS), maleimide-benzoyl-succinimide (MBS), gamma-maleimido-butyryloxy succinimide ester (GMBS), maleimido propionic acid (MPA) maleimido hexanoic acid (MHA), and maleimido undecanoic acid (MUA).

Primary amines are the principal targets for NHS esters. Accessible α-amine groups present on the N-termini of proteins and the ε-amine of lysine react with NHS esters. An amide bond is formed when the NHS ester conjugation reaction reacts with primary amines releasing N-

hydroxysuccinimide. These succinimide containing reactive groups are herein referred to as succinimidyl groups. In certain embodiments of the invention, the functional group on the protein will be a thiol group and the chemically reactive group will be a maleimido-containing group such as gamma-maleimide-butrylamide (GMBA or MPA). Such maleimide containing groups are referred to herein as maleido groups.

The maleimido group is most selective for sulfhydryl groups on peptides when the pH of the reaction mixture is 6.5-7.4. At pH 7.0, the rate of reaction of maleimido groups with sulfhydryls (e.g., thiol groups on proteins such as serum albumin or IgG) is 1000-fold faster than with amines. Thus, a stable thioether linkage between the maleimido group and the sulfhydryl can be formed.

In other embodiments, the linker includes at least one amino acid (e.g., a peptide of at least 2, 3, 4, 5, 6, 7, 10, 15, 20, 25, 40, or 50 amino acids). In certain embodiments, the linker is a single amino acid (e.g., any naturally occurring amino acid such as Cys). In other embodiments, a glycine-rich peptide such as a peptide having the sequence $[Gly\text{-}Gly\text{-}Gly\text{-}Gly\text{-}Ser]_n$ where n is 1, 2, 3, 4, 5 or 6 is used (SEQ ID NOS:169-174), as described in U.S. Patent No. 7,271,149. In other embodiments, a serine-rich peptide linker is used, as described in U.S. Patent No. 5,525,491. Serine rich peptide linkers include those of the formula $[X\text{-}X\text{-}X\text{-}X\text{-}Gly]_y$, where up to two of the X are Thr, and the remaining X are Ser, and y is 1 to 5 (e.g., Ser-Ser-Ser-Ser-Gly (SEQ ID NO:175), where y is greater than 1). In some cases, the linker is a single amino acid (e.g., any amino acid, such as Gly or Cys).

Examples of suitable linkers are succinic acid, Lys, Glu, and Asp, or a dipeptide such as Gly-Lys. When the linker is succinic acid, one carboxyl group thereof may form an amide bond with an amino group of the amino acid residue, and the other carboxyl group thereof may, for example, form an amide bond with an amino group of the peptide or substituent. When the linker is Lys, Glu, or Asp, the carboxyl group thereof may form an amide bond with an amino group of the amino acid residue, and the amino group thereof may, for

example, form an amide bond with a carboxyl group of the substituent. When Lys is used as the linker, a further linker may be inserted between the ε-amino group of Lys and the substituent. In one particular embodiment, the further linker is succinic acid, which, e.g., forms an amide bond with the ε-amino group of Lys and with an amino group present in the substituent. In one embodiment, the further linker is Glu or Asp (e.g., which forms an amide bond with the ε-amino group of Lys and another amide bond with a carboxyl group present in the substituent), that is, the substituent is an N^{ε}-acylated lysine residue.

Treatment of disease

As MT1-MMP has been linked to diseases including cancer, heart or vascular disease, and arthritis, the MT1-MMP inhibitors described herein and compositions including the inhibitors (e.g., pharmaceutical compositions) may be used to treat (e.g., prophylactically) any disease where MT1-MMP inhibition is desirable.

Exemplary cancers that can be treated according to the invention include cancers such as glioblastoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, schwannoma, meningioma, melanoma, neuroblastoma, retinoblastoma, leukemias (e.g., acute leukemia, acute lymphocytic leukemia, acute myelocytic leukemia, acute myeloblastic leukemia, acute promyelocytic leukemia, acute myelomonocytic leukemia, acute monocytic leukemia, acute erythroleukemia, chronic leukemia, chronic myelocytic leukemia, chronic lymphocytic leukemia), lung cancer (e.g., squamous cell carcinoma, adenocarinoma, or large cell carcinoma), colorectal cancer, ovarian cancer (e.g., ovarian adenocarcinoma), prostate cancer, polycythemia vera, lymphoma (Hodgkin's disease, non-Hodgkin's disease), Waldenstrom's macroglobulinemia, heavy chain disease, and solid tumors such as sarcomas and carcinomas (e.g., fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma,

endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilm's tumor, cervical cancer, uterine cancer, testicular cancer, lung carcinoma, small cell lung carcinoma, bladder carcinoma, and epithelial carcinoma. In some cases, the cancer is a brain cancer (e.g., glioblastoma, astrocytoma, glioma, medulloblastoma, and oligodendroma, neuroglioma, ependymoma, and meningioma).

Types of arthritis that may be treated using the compositions of the invention include osteoarthritis, rheumatoid arthritis, juvenile rheumatoid arthritis, psoriatic arthritis, polymyalgia rheumatica, and ankylosing spondylitis.

Heart and vascular diseases that may be treated using the compositions of the invention include hypertensive heart disease, atherosclerosis, restinosis, abdominal aortic aneurysm (AAA), thoracic aortic aneurysm, carotid artery disease, peripheral arterial disease (PAD), and renal artery disease.

Formulation, administration, and dosage

The present invention also features pharmaceutical compositions that contain a therapeutically effective amount of an MT1-MMP inhibitory compound of the invention. The composition can be formulated for use in a variety of drug delivery systems. One or more physiologically acceptable excipients or carriers can also be included in the composition for proper formulation. Suitable formulations for use in the present invention are found in *Remington's Pharmaceutical Sciences*, Mack Publishing Company,

Philadelphia, PA, 17th ed., 1985. For a brief review of methods for drug delivery, see, e.g., Langer (Science 249:1527-1533, 1990).

The pharmaceutical compositions are intended for parenteral, intranasal, topical, oral, or local administration, such as by a transdermal means, for prophylactic and/or therapeutic treatment. The pharmaceutical compositions can be administered parenterally (e.g., by intravenous, intramuscular, or subcutaneous injection), or by oral ingestion, or by topical application or intraarticular injection at areas affected by the vascular or cancer condition. Additional routes of administration include intravascular, intra-arterial, intratumor, intraperitoneal, intraventricular, intraepidural, as well as nasal, ophthalmic, intrascleral, intraorbital, rectal, topical, or aerosol inhalation administration. Sustained release administration is also specifically included in the invention, by such means as depot injections or erodible implants or components. Thus, the invention provides compositions for parenteral administration that comprise the above mention agents dissolved or suspended in an acceptable carrier, preferably an aqueous carrier, e.g., water, buffered water, saline, PBS, and the like. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions, such as pH adjusting and buffering agents, tonicity adjusting agents, wetting agents, detergents and the like. The invention also provides compositions for oral delivery, which may contain inert ingredients such as binders or fillers for the formulation of a tablet, a capsule, and the like. Furthermore, this invention provides compositions for local administration, which may contain inert ingredients such as solvents or emulsifiers for the formulation of a cream, an ointment, and the like.

These compositions may be sterilized by conventional sterilization techniques, or may be sterile filtered. The resulting aqueous solutions may be packaged for use as is, or lyophilized, the lyophilized preparation being combined with a sterile aqueous carrier prior to administration. The pH of the preparations typically will be between 3 and 11, between 5 and 9 or between 6 and 8 (e.g., between 7 and 8 or 7 to 7.5). The resulting compositions in solid

form may be packaged in multiple single dose units, each containing a fixed amount of the above-mentioned agent or agents, such as in a sealed package of tablets or capsules. The composition in solid form can also be packaged in a container for a flexible quantity, such as in a squeezable tube designed for a topically applicable cream or ointment.

The compositions containing an effective amount can be administered for prophylactic or therapeutic treatments. In prophylactic applications, compositions can be administered to a subject with a clinically determined predisposition or increased susceptibility to a disease associated with MT1-MMP activity. Compositions of the invention can be administered to the subject (e.g., a human) in an amount sufficient to delay, reduce, or preferably prevent the onset of clinical disease. In therapeutic applications, compositions are administered to a subject (e.g., a human) already suffering from disease in an amount sufficient to cure or at least partially arrest the symptoms of the condition and its complications. An amount adequate to accomplish this purpose is defined as a "therapeutically effective amount," an amount of a compound sufficient to substantially improve some symptom associated with a disease or a medical condition. For example, in prophylactic treatment of a disease associated with MT1-MMP activity (e.g., those described herein), an agent or compound that decreases, prevents, delays, suppresses, or arrests any symptom of the disease or condition would be therapeutically effective. A therapeutically effective amount of an agent or compound is not required to cure a disease or condition but will provide a treatment for a disease or condition such that the onset of the disease or condition is delayed, hindered, or prevented, or the disease or condition symptoms are ameliorated, or the term of the disease or condition is changed or, for example, is less severe or recovery is accelerated in an individual.

Effective amounts of the compositions described herein can depend on the severity of the disease or condition and the weight and general state of the patient, but generally range from about 0.05 μg to about 1 g (e.g., 0.5-100 mg) of an equivalent amount of the agent or agents per dose per patient. Suitable

regimes for initial administration and booster administrations are typified by an initial administration followed by repeated doses at one or more hourly, daily, weekly, or monthly intervals by a subsequent administration. The total effective amount of an agent present in the compositions of the invention can be administered to a mammal as a single dose, either as a bolus or by infusion over a relatively short period of time, or can be administered using a fractionated treatment protocol, in which multiple doses are administered over a more prolonged period of time (e.g., a dose every 4-6, 8-12, 14-16, or 18-24 hours, or every 2-4 days, 1-2 weeks, once a month). Alternatively, continuous intravenous infusion sufficient to maintain therapeutically effective concentrations in the blood are contemplated.

The therapeutically effective amount of one or more agents present within the compositions of the invention and used in the methods of this invention applied to mammals (e.g., humans) can be determined by the ordinarily-skilled artisan with consideration of individual differences in age, weight, and the condition of the mammal. The agents of the invention are administered to a subject (e.g. a mammal, such as a human) in an effective amount, which is an amount that produces a desirable result in a treated subject (e.g. reduction in MT1-MMP activity). Therapeutically effective amounts can also be determined empirically by those of skill in the art.

The patient may also receive an agent in the range of about 0.05 to 1 g dose one or more times per week (e.g., 2, 3, 4, 5, 6, or 7 or more times per week), 0.1 to 2,500 (e.g., 2,000, 1,500, 1,000, 500, 100, 10, 1, 0.5, or 0.1) μg dose per week. A patient may also receive an agent of the composition in the range of 0.1 to 3,000 μg per dose once every two or three weeks.

Single or multiple administrations of the compositions of the invention comprising an effective amount can be carried out with dose levels and pattern being selected by the treating physician. The dose and administration schedule can be determined and adjusted based on the severity of the disease or condition in the patient, which may be monitored throughout the course of

treatment according to the methods commonly practiced by clinicians or those described herein.

The compounds of the present invention may be used in combination with either conventional methods of treatment or therapy or may be used separately from conventional methods of treatment or therapy.

When the compounds of this invention are administered in combination therapies with other agents, they may be administered sequentially or concurrently to an individual. Alternatively, pharmaceutical compositions according to the present invention may be comprised of a combination of a compound of the present invention in association with a pharmaceutically acceptable excipient, as described herein, and another therapeutic or prophylactic agent known in the art.

Combination therapy

The compositions of the invention may be formulated or administered with any other therapy regimen known in the art. For example, when a composition of the invention is used to treat cancer, the composition may be administered in conjunction with any antiproliferative agent known in the art (e.g., those described herein).

Additional therapeutics for treating cancer include those shown in Table 2 below.

TABLE 2

Alkylating agents	cyclophosphamide busulfan ifosfamide melphalan hexamethylmelamine thiotepa chlorambucil dacarbazine carmustine	lomustine procarbazine altretamine estramustine phosphate mechlorethamine streptozocin temozolomide semustine

PATENT
ATTORNEY DOCKET NO. V82819WO

TABLE 2

Platinum agents	cisplatin oxaliplatin spiroplatinum carboxyphthalatoplatinum tetraplatin ormiplatin iproplatin	carboplatinum ZD-0473 (AnorMED) lobaplatin (Aeterna) satraplatin (Johnson Matthey) BBR-3464 (Hoffmann-La Roche) SM-11355 (Sumitomo) AP-5280 (Access)
Antimetabolites	azacytidine gemcitabine capecitabine 5-fluorouracil floxuridine 2-chlorodeoxyadenosine 6-mercaptopurine 6-thioguanine cytarabin 2-fluorodeoxy cytidine methotrexate idatrexate tomudex	trimetrexate deoxycoformycin fludarabine pentostatin raltitrexed hydroxyurea decitabine (SuperGen) clofarabine (Bioenvision) irofulven (MGI Pharma) DMDC (Hoffmann-La Roche) ethynylcytidine (Taiho)
Topoisomerase inhibitors	amsacrine epirubicin etoposide epirubicin teniposide or mitoxantrone irinotecan 7-ethyl-10-hydroxy-camptothecin topotecan dexrazoxanet (TopoTarget) pixantrone (Novuspharma) rebeccamycin analogue (Exelixis) BBR-3576 (Novuspharma) rubitecan (SuperGen)	exatecan mesylate (Daiichi) quinamed (ChemGenex) gimatecan (Sigma-Tau) diflomotecan (Beaufour-Ipsen) TAS-103 (Taiho) elsamitrucin (Spectrum) J-107088 (Merck & Co) BNP-1350 (BioNumerik) CKD-602 (Chong Kun Dang) KW-2170 (Kyowa Hakko)
Antitumor antibiotics	dactinomycin (actinomycin D) doxorubicin (adriamycin) deoxyrubicin valrubicin daunorubicin (daunomycin) therarubicin idarubicin rubidazone plicamycinp porfiromycin cyanomorpholinodoxorubicin mitoxantrone (novantrone)	amonafide azonafide anthrapyrazole oxantrazole losoxantrone bleomycin sulfate (blenoxane) bleomycinic acid bleomycin A bleomycin B mitomycin C MEN-10755 (Menarini) GPX-100 (Gem Pharmaceuticals)

PATENT
ATTORNEY DOCKET NO. V82819WO

TABLE 2

Antimitotic agents	paclitaxel docetaxel colchicine vinblastine vincristine vinorelbine vindesine dolastatin 10 (NCI) rhizoxin (Fujisawa) mivobulin (Warner-Lambert) cemadotin (BASF) RPR 109881A (Aventis) TXD 258 (Aventis) epothilone B (Novartis) T 900607 (Tularik) T 138067 (Tularik) cryptophycin 52 (Eli Lilly) vinflunine (Fabre) auristatin PE (Teikoku Hormone) BMS 247550 (BMS) BMS 184476 (BMS) BMS 188797 (BMS) taxoprexin (Protarga)	SB 408075 (GlaxoSmithKline) E7010 (Abbott) PG-TXL (Cell Therapeutics) IDN 5109 (Bayer) A 105972 (Abbott) A 204197 (Abbott) LU 223651 (BASF) D 24851 (ASTAMedica) ER-86526 (Eisai) combretastatin A4 (BMS) isohomohalichondrin-B (PharmaMar) ZD 6126 (AstraZeneca) PEG-paclitaxel (Enzon) AZ10992 (Asahi) IDN-5109 (Indena) AVLB (Prescient NeuroPharma) azaepothilone B (BMS) BNP-7787 (BioNumerik) CA-4 prodrug (OXiGENE) dolastatin-10 (NIH) CA-4 (OXiGENE)
Aromatase inhibitors	aminoglutethimide letrozole anastrazole formestane	exemestane atamestane (BioMedicines) YM-511 (Yamanouchi)
Thymidylate synthase inhibitors	pemetrexed (Eli Lilly) ZD-9331 (BTG)	nolatrexed (Eximias) CoFactor™ (BioKeys)
DNA antagonists	trabectedin (PharmaMar) glufosfamide (Baxter International) albumin + 32P (Isotope Solutions) thymectacin (NewBiotics) edotreotide (Novartis)	mafosfamide (Baxter International) apaziquone (Spectrum Pharmaceuticals) O6 benzyl guanine (Paligent)
Farnesyltransferase inhibitors	arglabin (NuOncology Labs) lonafarnib (Schering-Plough) BAY-43-9006 (Bayer)	tipifarnib (Johnson & Johnson) perillyl alcohol (DOR BioPharma)
Pump inhibitors	CBT-1 (CBA Pharma) tariquidar (Xenova) MS-209 (Schering AG)	zosuquidar trihydrochloride (Eli Lilly) biricodar dicitrate (Vertex)
Histone acetyltransferase inhibitors	tacedinaline (Pfizer) SAHA (Aton Pharma) MS-275 (Schering AG)	pivaloyloxymethyl butyrate (Titan) depsipeptide (Fujisawa)
Metalloproteinase inhibitors	Neovastat (Aeterna Laboratories) marimastat (British Biotech)	CMT-3 (CollaGenex) BMS-275291 (Celltech)
Ribonucleoside reductase inhibitors	gallium maltolate (Titan) triapine (Vion)	tezacitabine (Aventis) didox (Molecules for Health)
TNF alpha agonists/antagonists	virulizin (Lorus Therapeutics) CDC-394 (Celgene)	revimid (Celgene)
Endothelin A receptor antagonist	atrasentan (Abbott) ZD-4054 (AstraZeneca)	YM-598 (Yamanouchi)
Retinoic acid receptor agonists	fenretinide (Johnson & Johnson) LGD-1550 (Ligand)	alitretinoin (Ligand)

PATENT
ATTORNEY DOCKET NO. V82819WO

TABLE 2

Immuno-modulators	interferon oncophage (Antigenics) GMK (Progenics) adenocarcinoma vaccine (Biomira) CTP-37 (AVI BioPharma) IRX-2 (Immuno-Rx) PEP-005 (Peplin Biotech) synchrovax vaccines (CTL Immuno) melanoma vaccine (CTL Immuno) p21 RAS vaccine (GemVax) cepharanthine	dexosome therapy (Anosys) pentrix (Australian Cancer Technology) ISF-154 (Tragen) cancer vaccine (Intercell) norelin (Biostar) BLP-25 (Biomira) MGV (Progenics) ß-alethine (Dovetail) CLL therapy (Vasogen)
Hormonal and antihormonal agents	estrogens conjugated estrogens ethinyl estradiol chlortrianisen idenestrol hydroxyprogesterone caproate medroxyprogesterone testosterone testosterone propionate; fluoxymesterone methyltestosterone diethylstilbestrol megestrol Tamoxifen toremofine dexamethasone prednisone	methylprednisolone prednisolone aminoglutethimide leuprolide goserelin leuporelin bicalutamide flutamide octreotide nilutamide mitotane P-04 (Novogen) 2-methoxyestradiol (EntreMed) arzoxifene (Eli Lilly) raloxifene
Photodynamic agents	talaporfin (Light Sciences) Theralux (Theratechnologies) motexafin gadolinium (Pharmacyclics)	Pd-bacteriopheophorbide (Yeda) lutetium texaphyrin (Pharmacyclics) hypericin
Tyrosine Kinase Inhibitors	imatinib leflunomide (Sugen/Pharmacia) ZD1839 (AstraZeneca) erlotinib (Oncogene Science) gefitinib canertinib (Pfizer) squalamine (Genaera) SU5416 (Pharmacia) SU6668 (Pharmacia) ZD4190 (AstraZeneca) ZD6474 (AstraZeneca) vatalanib (Novartis) PKI166 (Novartis) GW2016 (GlaxoSmithKline) EKB-509 (Wyeth) EKB-569 (Wyeth)	kahalide F (PharmaMar) CEP-701 (Cephalon) CEP-751 (Cephalon) MLN518 (Millenium) PKC412 (Novartis) phenoxodiol () trastuzumab (Genentech) C225 (ImClone) rhu-Mab (Genentech) MDX-H210 (Medarex) 2C4 (Genentech) MDX-447 (Medarex) ABX-EGF (Abgenix) IMC-1C11 (ImClone)

PATENT
ATTORNEY DOCKET NO. V82819WO

TABLE 2

Miscellaneous agents	
SR-27897 (CCK A inhibitor, Sanofi-Synthelabo)	BCX-1777 (PNP inhibitor, BioCryst)
tocladesine (cyclic AMP agonist, Ribapharm)	ranpirnase (ribonuclease stimulant, Alfacell)
alvocidib (CDK inhibitor, Aventis)	galarubicin (RNA synthesis inhibitor, Dong-A)
CV-247 (COX-2 inhibitor, Ivy Medical)	tirapazamine (reducing agent, SRI International)
P54 (COX-2 inhibitor, Phytopharm)	N-acetylcysteine (reducing agent, Zambon)
CapCell™ (CYP450 stimulant, Bavarian Nordic)	R-flurbiprofen (NF-kappaB inhibitor, Encore)
GCS-100 (gal3 antagonist, GlycoGenesys)	3CPA (NF-kappaB inhibitor, Active Biotech)
G17DT immunogen (gastrin inhibitor, Aphton)	seocalcitol (vitamin D receptor agonist, Leo)
efaproxiral (oxygenator, Allos Therapeutics)	131-I-TM-601 (DNA antagonist, TransMolecular)
PI-88 (heparanase inhibitor, Progen)	eflornithine (ODC inhibitor , ILEX Oncology)
tesmilifene (histamine antagonist, YM BioSciences)	minodronic acid (osteoclast inhibitor, Yamanouchi)
histamine (histamine H2 receptor agonist, Maxim)	indisulam (p53 stimulant, Eisai)
tiazofurin (IMPDH inhibitor, Ribapharm)	aplidine (PPT inhibitor, PharmaMar)
cilengitide (integrin antagonist, Merck KGaA)	rituximab (CD20 antibody, Genentech)
SR-31747 (IL-1 antagonist, Sanofi-Synthelabo)	gemtuzumab (CD33 antibody, Wyeth Ayerst)
CCI-779 (mTOR kinase inhibitor, Wyeth)	PG2 (hematopoiesis enhancer, Pharmagenesis)
exisulind (PDE V inhibitor, Cell Pathways)	Immunol™ (triclosan oral rinse, Endo)
CP-461 (PDE V inhibitor, Cell Pathways)	triacetyluridine (uridine prodrug , Wellstat)
AG-2037 (GART inhibitor, Pfizer)	SN-4071 (sarcoma agent, Signature BioScience)
WX-UK1 (plasminogen activator inhibitor, Wilex)	TransMID-107™ (immunotoxin, KS Biomedix)
PBI-1402 (PMN stimulant, ProMetic LifeSciences)	PCK-3145 (apoptosis promoter, Procyon)
bortezomib (proteasome inhibitor, Millennium)	doranidazole (apoptosis promoter, Pola)
SRL-172 (T cell stimulant, SR Pharma)	CHS-828 (cytotoxic agent, Leo)
TLK-286 (glutathione S transferase inhibitor, Telik)	trans-retinoic acid (differentiator, NIH)
PT-100 (growth factor agonist, Point Therapeutics)	MX6 (apoptosis promoter, MAXIA)
midostaurin (PKC inhibitor, Novartis)	apomine (apoptosis promoter, ILEX Oncology)
bryostatin-1 (PKC stimulant, GPC Biotech)	urocidin (apoptosis promoter, Bioniche)
CDA-II (apoptosis promoter, Everlife)	Ro-31-7453 (apoptosis promoter, La Roche)
SDX-101 (apoptosis promoter, Salmedix)	brostallicin (apoptosis promoter, Pharmacia)
ceflatonin (apoptosis promoter, ChemGenex)	

Additional therapeutics for treating a vascular or heart disease include agents such as anti-inflammatory agents, e.g., non-steroidal anti-inflammatory drugs (NSAIDs; e.g., detoprofen, diclofenac, diflunisal, etodolac, fenoprofen,

flurbiprofen, ibuprofen, indomethacin, ketoprofen, meclofenameate, mefenamic acid, meloxicam, nabumeone, naproxen sodium, oxaprozin, piroxicam, sulindac, tolmetin, celecoxib, rofecoxib, aspirin, choline salicylate, salsalte, and sodium and magnesium salicylate) steroids (e.g., cortisone, dexamethasone, hydrocortisone, methylprednisolone, prednisolone, prednisone, triamcinolone), antibacterial agents (e.g., azithromycin, clarithromycin, erythromycin, roxythromycin, gatifloxacin, levofloxacin, amoxicillin, or metronidazole), platelet aggregation inhibitors (e.g., abciximab, aspirin, cilostazol, clopidogrel, dipyridamole, eptifibatide, ticlopidine, or tirofiban), anticoagulants (e.g., dalteparin, danaparoid, enoxaparin, heparin, tinzaparin, or warfarin), antipyretics (e.g., acetaminophen), ticlopidine, clopidogrel, angiotensin converting enzyme inhibitors, beta blockers, pentoxifylline, cilostazol, estrogen replacement therapy, lipid-lowering agents (e.g., cholestyramine, colestipol, nicotinic acid, gemfibrozil, probucol, ezetimibe, or statins such as atorvastatin, rosuvastatin, lovastatin simvastatin, pravastatin, cerivastatin, and fluvastatin).

Additional therapeutics for treating rheumatoid arthritis include CD20 binding antibodies (e.g., rituximab, ocrelizumab, ofatumumab, HuMax-CD20, and variants thereof), DMARDS (disease-modifying anti-rheumatic drugs), NSAIDs (non-steroidal anti-inflammatory drugs), immunosuppressants (e.g., azathioprine; mycophenolate mofetil (CellCept®; Roche)), analgesics, corticosteroids (e.g., prednisone), glucocorticosteroids, cyclophosphamides, HUMIRA™ (adalimumab; Abbott Laboratories), ARAVA® (leflunomide), REMICADE® (infliximab; Centocor, Inc.), ENBREL (etanercept; Amgen), ACTEMRA (tocilizumab; Roche, Switzerland), and COX-2 inhibitors (e.g., GW406381). DMARDs include methotrexate, hydroxycloroquine, sulfasalazine, leflunomide, etanercept, infliximab, azathioprine, D-penicillamine, gold or gold salts (oral or intramuscular), minocycline, cyclosporine, cyclosporine A, Staphylococcal protein A immunoadsorptionm and interleukin-1 blockers (e.g., anakinra and interleukin-1 receptor antagonist). The DMARD may be a TNFα blocker (e.g., adalimumab (human

monoclonal anti-TNFα antibody), CDP870 (UCB), pegsunercept, and atacicept), infliximab (chimeric monoclonal anti-TNFα antibody), and etanercept ("immunoadhesin" fusion protein consisting of the extracellular ligand binding portion of the human 75 kD (p75) tumor necrosis factor receptor (TNFR) and the Fc portion of a human IgG1). ACTEMRA (tocilizumab) is a humanized anti-human interleukin-6 (IL-6) receptor. NSAIDs include acetaminophen, ibuprofen, aspirin, an opiate, or lidocaine (topical). Other agents used to treat rheumatoid arthritis include pioglitazone, canakinumab, p38 kinase inhibitors (e.g., SCIO-469, VX-702, BMS-582949, and PH-797804), MEK inhibitors (e.g., ARRY-438162), rosiglitazone, Tripterygium wilfordi Hook F, SBI-087, Cura-100, AZD5672 (AstraZeneca), paclitaxel, lumiracoxib, golimumab, estrogen receptor-β agonist (e.g., ERB-041), A3 adenosine receptor agonist (e.g,. CF101; Can-Fite BioPharma), roxithromycin, ADL5859 (Adolor Corp.), GW856553 (GlaxoSmithKline), ASK8007 (Astellas Pharma, Inc.), HE3286 (Hollis-Eden Pharmaceuticals, San Diego, Calif.), TRU-015 (Wyeth), belimumab, AZD9056 (AstraZeneca), ACZ885 (Novartis), GSK3152314A (GlaxoSmithKline), anti-IL-17 antibodies (e.g., AIN457 (Novartis) and AMG 827 (Amgen)), Fentanyl transdermal patch (Janssen Pharmaceutica N.V., Belgium), valdecoxib, CNTO 136 (Centocor, Inc.), imatinib (Novartis), JAK-3 inhibitors (e.g., CP-690,550 (Pfizer)), cathepsin-S inhibitors (e.g., RWJ-445380 (Johnson & Johnson)), ISIS 104838 (Isis Pharmaceuticals), MM-093 (Merrimack Pharmaceuticals), bovine type II collagen, lovastatin, anti-RANK ligand antibodies (e.g., denosumab (Amgen)), dnaj peptide, Etoricoxib (Merck), SB-681323 (GlaxoSmithKline), Rofecoxib (Merck), omegaven, anti-CD19 antibodies (e.g., MDX-1342 (Medarex)), AMG 108 (Amgen), TMI-005 (Wyeth), Abatacept (Bristol-Myers-Squibb), baminercept (Biogen-Idec), fostamatinib disodium (Rigel Pharmaceuticals), temsirolimus (Wyeth), ARRY-371797 (Array BioPharma), Natalizumab (Elan Pharmaceuticals), AMG 719 (Amgen), CE-224,535 (Pfizer), TAK-715 (Takeda), TAK-783 (Takeda), BG9924 (Biogen Idec), GW274150 (GlaxoSmithKline), GSK1827771 (GlaxoSmithKline), CH-1504 (Chelsea

Therapeutics), Certolizumab pegol (UCB), tramadol, LY2127399 (Eli Lily), curcumin, MTRX1011A (Tolerex/Genetech), AMG 714 (Amgen), CAM-3001 (MedImmune), BIIB023 (Biogen Idec), SSR150106 (Sanofi-Aventis), STA 5326 (Synta Pharmaceuticals), P38 Inhibitor (4) (Hoffman-La Roche), etoricoxib (Merck), MEDI-522 (MedImmune), γ-linolenic acid, Ramipril (Sanofi-Aventis), CRx-102 (CombinatoRx), efalizumab, LY2189102 (Eli Lily), MK-0873 (Merck), fontolizumab (PDL BioPharma), Maraviroc (Pfizer), HuMax-CD4 (Genmab), CP-195,543 (Pfizer), meloxicam (Boehringer Ingelheim Pharmaceuticals), bucillamine, PD 0360324 (Pfizer), FANG(30), PLA-695 (Wyeth), PG-760564 (Procter and Gamble), MK0812 (Merck), tgAAC94 (Targeted Genetics), SMP-114 (Dainippon Sumitomo Pharma Europe), RhuDex (Medigene), MK0359 (Merck). For conventional treatment of RA, see, e.g., "Guidelines for the management of rheumatoid arthritis" *Arthritis & Rheumatism* 46:328-346 (2002).

These additional therapeutic agents may be administered within 14 days, 7 days, 2 days, 1 day, 12 hours, 6 hours, or 1 hour of administration of a MT1-MMP inhibitory therapeutic, or simultaneously therewith. The additional therapeutic agent may be present in the same or different pharmaceutical compositions as the MT1-MMP inhibitory therapeutic of the invention. When present in different pharmaceutical compositions, different routes of administration may be used. For example, the MT1-MMP inhibitory therapeutic may be administered orally, while a second agent may be administered by intravenous, intramuscular, or subcutaneous injection.

The following examples are intended to illustrate, rather than limit, the invention

Example 1

Generation of MT1-MMP derived peptides

In performing the experiments described in the examples below, the following peptides were generated. First, a peptide having the sequence of the

cytoplasmic domain of MT1-MMP (RRHGTPRRLLYCQRSLLDKV; SEQ ID NO:117), as well as a nonphosphorylatable form of this peptide (RRHGTPRRLLFCQRSLLDKV; SEQ ID NO:118), which has a phenylalanine in place of a tyrosine at the position corresponding to amino acid 573 of the human MT1-MMP sequence were generated. The nonphosphorylatable peptide has been termed "M-14."

In addition, peptides having the M-14 sequence fused to the cell penetrating third helix of the homeodomain of Antennapedia protein (RQIKIWFQNRRMKWKK; SEQ ID NO:119) were generated. This fusion peptide is called ACM-14 and has the sequence Biotin-Ahx-RQIKIWFQNRRMKWKK-RRHGTPRRLLFCQRSLLDKV (SEQ ID NO:176). For use as a control, a version of the fusion peptide, where the cytoplamsic MT1-MMP domain sequence is scrambled was generated. This peptide has the sequence Biotin-Ahx-RQIKIWFQNRRMKWKK-TLRQRRCLPHFDSGLRKVRL (SEQ ID NO:177) and is termed scACM-14. These peptides are shown in Figure 1.

Example 2

Expression of Y573F MT1-MMP inhibits tumor growth

HT1080 fibrosarcoma cells were stably transfected with either WT MT1-MMP or Y573F MT1-MMP. HT1080 cells are very aggressive cancer cells that express elevated levels of MT1-MMP. Both groups of cells were transplanted subcutaneously into athymic nude mice, and tumor growth was monitored. As shown in Figure 2, cells expressing the cytoplasmic domain of MT1-MMP exhibited significant tumor growth, whereas no tumor growth was observed in the mice that received cells expressing Y573F mutant.

Example 3

ACM-14 and scACM-14 are efficiently taken up by fibrosacrcoma cells

To determine whether ACM-14 and scACM-14 were capable of entering tumor cells, HT-1080 fibrosarcoma cells were incubated with each peptide (1

μM) for one hour, and peptide uptake was analyzed by immunofluorescence and confocal microscopy. As shown in Figure 3, both ACM-14 and its scrambled version (scACM-14) were visualized in the cells, indicated efficient and rapid cellular uptake.

Example 4

Selective inhibition of MT1-MMP phosphorylation

We have shown that overexpression of the Y573F mutant of full length MT1-MMP in fibrosarcoma cells reduces the Src-mediated phosphorylation of endogenous MT1-MMP (Nyalendo et al., J Biol Chem 282, 15690-15699, 2007). In order to determine the effect of ACM-14 in this process, we have performed an in vitro phosphorylation assay. The GST-MT1-MMP cytoplasmic tail fusion protein (GST-MT) was incubated with recombinant Src kinase in the presence of ATP and the phosphorylation products were analyzed by western blotting. As shown in Figure 4, Src-mediated tyrosine phosphorylation of GST-MT was completely inhibited by incubation with ACM-14. The inhibition was not observed with the control peptide scACM-14, indicating that ACM-14 selectively inhibits MT1-MMP phosphorylation in vitro. These surprising results suggest that a nonphosphorylatable form of the cytoplasmic domain of MT1-MMP alone is sufficient to inhibit MT1-MMP phosphorylation in the absence of other portions of the MT1-MMP protein.

Example 5

Inhibition of tumor cell proliferation within 3D collagen matrix

The effect of ACM-14 on tumor cell proliferation under planar conditions (2D) and tumorigenic conditions (3D) was evaluated. Different tumor cell lines were incubated with the peptides and grown atop of a collagen film (2D) or within a collagen gel (3D). As presented in Figure 5, ACM-14 does not affect tumor cell proliferation in 2D conditions. By contrast, ACM-14 significantly reduces the proliferation of several tumor cells grown in 3D collagen gels. Indeed, treatment of human brain cancer cells with ACM-14

decreases their proliferation in 3D matrix (50% reduction for glioblastoma cells and 35% for medulloblastoma cells), whereas treatment with the scrambled peptide (scACM-14) has no effect on the proliferation of these cells. ACM-14 also inhibits 3D proliferation of prostate cancer, breast cancer, bone cancer, and fibrosarcoma cells.

Example 6

Inhibition of tumor growth *in vivo*

The effects of ACM-14 on xenograft tumor formation in vivo were also investigated. Fibrosarcoma cells were implanted subcutaneously into the flank of athymic nude mice. Tumors were allowed to grow until approximately 100 mm^3 before the mice were randomly separated into three groups for treatment (vehicle, scACM-14, and ACM-14). Mice were given daily subcutaneous administrations of 10 mg/kg of peptides or vehicle. ACM-14 strongly reduces tumor growth by 70% 26 days post-implantation (Figure 6). The survival of mice that have received ACM-14 was dramatically increased by 10 days (Figure 7). Furthermore, 17% of tumors completely regressed and totally disappeared following ACM-14 administration.

Example 7

Angiopep-M14 conjugate

For delivery of the M14 peptide across the BBB, a fusion peptide containing the M14 sequence and an Angiopep peptide sequence (e.g., SEQ ID NO:178) is produced. In this example, the Angiopep peptide has the sequence of Angiopep-2, which is capable of crossing the BBB of a mammalian subject (Figure 8).

Other embodiments

All patents; patent applications, including U.S. Provisional Application No. 61/138,375, filed December 17, 2008; and publications mentioned in this specification are herein incorporated by reference to the same extent as if each

independent patent, patent application, or publication was specifically and individually indicated to be incorporated by reference.

What is claimed is:

PATENT
ATTORNEY DOCKET NO. V82819WO

CLAIMS

1. A composition comprising a soluble polypeptide comprising an amino acid sequence substantially identical to the cytoplasmic domain of membrane-type 1 matrix metalloproteinase (MT1-MMP) or a fragment thereof, wherein said polypeptide is capable of inhibiting MT1-MMP activity.

2. The composition of claim 1, wherein said polypeptide is selectively inhibits MT1-MMP activity.

3. The composition of claim 1, wherein said amino acid sequence lacks a phosphorylatable tyrosine at the amino acid position corresponding to position 573 of the human MT1-MMP sequence.

4. The composition of claim 3, wherein said amino acid sequence has a substitution, deletion, or modification at said position 573.

5. The composition of claim 4, wherein said substitution is phenylalanine.

6. The composition of any of claims 1-5, wherein said amino acid sequence has 0, 1, 2, 3, 4, 5, 6, or 7 substitutions as compared to the sequence of the cytoplasmic domain of human MT1-MMP.

7. The composition of any of claims 1-6, wherein said amino acid sequence is at least 80% identical to the sequence of cytoplasmic domain of human MT1-MMP, or the cytoplasmic domain of human MT1-MMP having a deletion, substitution, or modification at the position corresponding to position 573 of the human MT1-MMP sequence.

8. The composition of any of claims 1-7, wherein said amino acid sequence is RRHGTPRRLLFCQRSLLDKV (SEQ ID NO:118), or an MT1-MMP inhibitory fragment thereof.

9. The composition of any of claims 1-8, wherein said polypeptide further comprises an amino acid sequence capable of penetrating a cellular membrane, capable of entering a particular cell type, or capable of crossing the blood-brain barrier (BBB).

10. The composition of claim 9, wherein said amino acid sequence capable of penetrating a cellular membrane is substantially identical to a polypeptide selected from the group consisting of the third helix of the homeodomain of the antennapedia protein (SEQ ID NO:119), the antennapedia leader peptide (CT) (SEQ ID NO:120), the antennapedia peptide amide (SEQ ID NO:121), Cys(Npys)-antennapedia peptide amide (SEQ ID NO:122), a cytoplasmic transduction peptide (CTP), HSV-1 VP22, $(Arg)_9$ (SEQ ID NO:137), Cys(Npys)-$(Arg)_9$ (SEQ ID NO:138), Cys(Npys)-$(D\text{-}Arg)_9$, [Cys58]105Y cell penetrating peptide (SEQ ID NO:139), peptide 105Y (SEQ ID NO:140), buforin (SEQ ID NO:141), chimeric rabies virus glycoprotein fragment (RVG-9R; SEQ ID NO:142), Cys(Npys)-TAT(47-57) (SEQ ID NO:143), Cys-TAT(47-57) (SEQ ID NO:144), lipid membrane translocating peptide (SEQ ID NO:145), D-isomer-lipid membrane translocating peptide, mastoparan (SEQ ID NO:146), mastoparan 7 (SEQ ID NO:147), mastoparan X (SEQ ID NO:148), MEK1 derived peptide inhibitor 1 (SEQ ID NO:149), myristoyl-MEK1 derived peptide inhibitor 1 (SEQ ID NO:150), stearyl-MEK-1 derived peptide inhibitor 1 amide (SEQ ID NO:151), membrane-permeable sequence (SEQ ID NO:152), HIV related MPG ((SEQ ID NO:153), aminopeptidase N ligand (CD13), NGR peptide (SEQ ID NO:154), NGR peptide 1, NGR peptide 2 (SEQ ID NO:155), NGR peptide 3 (SEQ ID NO:156), NGR peptide 4, Pep-1 (Chariot™; SEQ ID NO:157), SynB1 (SEQ ID NO:158), biotin-TAT(47-57) (SEQ ID NO:159), TAT(47-57) (SEQ ID

NO:160), TAT(47-57) GGG-Cys(Npys) (SEQ ID NO:161), TAT(48-57) (SEQ ID NO:162), Tat-C(48-57) (SEQ ID NO:163), transdermal peptide (SEQ ID NO:164), transportan (SEQ ID NO:165), and transportan 10 (SEQ ID NO:166).

11. The composition of claim 10, wherein said amino acid sequence capable of penetrating a cell membrane is the third helix of the homeodomain of the antennapedia protein (RQIKIWFQNRRMKWKK; SEQ ID NO:119).

12. The composition of claim 11, wherein said polypeptide comprises the sequences RQIKIWFQNRRMKWKK (SEQ ID NO:119) and RRHGTPRRLLFCQRSLLDKV (SEQ ID NO:118).

13. The composition of claim 12, wherein said polypeptide comprises the sequence RQIKIWFQNRRMKWKKRRHGTPRRLLFCQRSLLDKV (SEQ ID NO:176).

14. The composition of claim 9, wherein said amino acid sequence capable of crossing the BBB is at least 90% identical to Angiopep-2 (SEQ ID NO:97) or Angiopep-1 (SEQ ID NO:67).

15. The composition of claim 14, wherein said amino acid sequence capable of crossing the BBB is Angiopep-1 (SEQ ID NO:67) or Angiopep-2 (SEQ ID NO:97).

16. The composition of claim 15, wherein said polypeptide comprises both Angiopep-2 (SEQ ID NO:97) and the sequence RRHGTPRRLLFCQRSLLDKV (SEQ ID NO:118).

17. The composition of claim 16, wherein said polypeptide comprises the sequence TFFYGGSRGKRNNFKTEEYRRHGTPRRLLFCQRSLLDKV (SEQ ID NO:178).

PATENT
ATTORNEY DOCKET NO. V82819WO

18. The composition of claim 9, wherein amino acid sequence capable of entering a particular cell type is at least 90% identical to Angiopep-7 (SEQ ID NO:112).

19. The composition of claim 18, wherein said amino acid sequence capable of entering a particular cell type is Angiopep-7 (SEQ ID NO:112).

20. The composition of any of claims 1-19, wherein said composition is a liposomal formulation.

21. The composition of claim 20, wherein said liposome comprises a peptide vector, wherein said vector peptide is on the exterior surface of said liposome.

22. The composition any of claims 1-21, wherein said composition is formulated with a pharmaceutically acceptable carrier.

23. A method of reducing MT1-MMP phosphorylation in a cell, said method comprising administering a composition of any of claims 1-22 to said cell.

24. The method of claim 23, wherein said cell is in a subject.

25. A method of treating a disease characterized by increased MT1-MMP activity, said method comprising administering a composition of any of claims 1-22 to said subject in an amount sufficient to treat said disease.

26. The method of claim 25, wherein said disease is selected from the group consisting of a cancer, heart or vascular disease, and arthritis.

PATENT
ATTORNEY DOCKET NO. V82819WO

27. A method of treating a subject having a cancer, said method comprising administering a composition of any of claims 1-22 to said subject in an amount sufficient to treat said cancer.

28. The method of claim 27, wherein said cancer is selected from the group consisting of brain cancer, acute leukemia, acute lymphocytic leukemia, acute myelocytic leukemia, acute myeloblastic leukemia, acute promyelocytic leukemia, acute myelomonocytic leukemia, acute monocytic leukemia, acute erythroleukemia, chronic leukemia, chronic myelocytic leukemia, chronic lymphocytic leukemia, polycythemia vera, Hodgkin's disease, non-Hodgkin's disease, Waldenstrom's macroglobulinemia, heavy chain disease, fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilm's tumor, cervical cancer, uterine cancer, testicular cancer, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, craniopharyngioma, pinealoma, hemangioblastoma, acoustic neuroma, schwannoma, melanoma, neuroblastoma, retinoblastoma, lung cancer, squamous cell carcinoma, adenocarcinoma, large cell carcinoma, and colon cancer.

29. The method of claim 28, wherein said brain cancer is glioblastoma, astrocytoma, glioma, medulloblastoma, oligodendroma, neuroglioma, ependymoma, or meningioma.

30. A method of treating a subject having heart disease or vascular disease, said method comprising administering a composition of any of claims 1-22 to said subject in an amount sufficient to treat said disease.

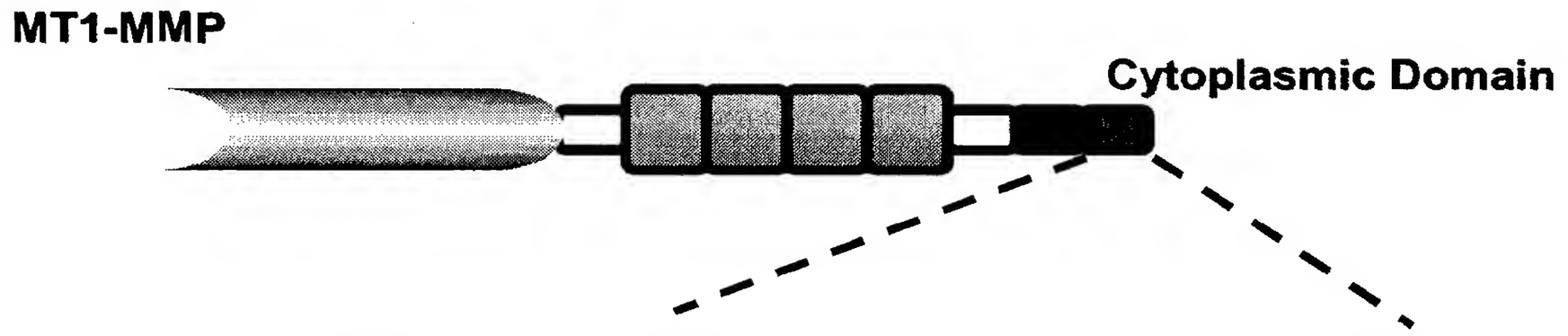
31. The method of claim 30, wherein said vascular disease is selected from the group consisting of atherosclerosis, restinosis, abdominal aortic aneurysm, thoracic aortic aneurysm, carotid artery disease, peripheral arterial disease, and renal artery disease.

32. The method of claim 30, wherein said heart disease is hypertensive heart disease.

33. A method of treating a subject having arthritis, said method comprising administering a composition of any of claims 1-22 to said subject in an amount sufficient to treat said arthritis.

34. The method of claim 33, wherein said arthritis is osteoarthritis or rheumatoid arthritis.

35. The method of any of claims 24-34, wherein said subject is a human.


MT1-MMP
Cytoplasmic Domain
Wild-Type (WT)
63RRHGTPRRLLYCQRSLLDKV582
Mutant (Y573F) (M14)
563RRHGTPRRLLFCQRSLLDKV582
(non-phosporylatable)

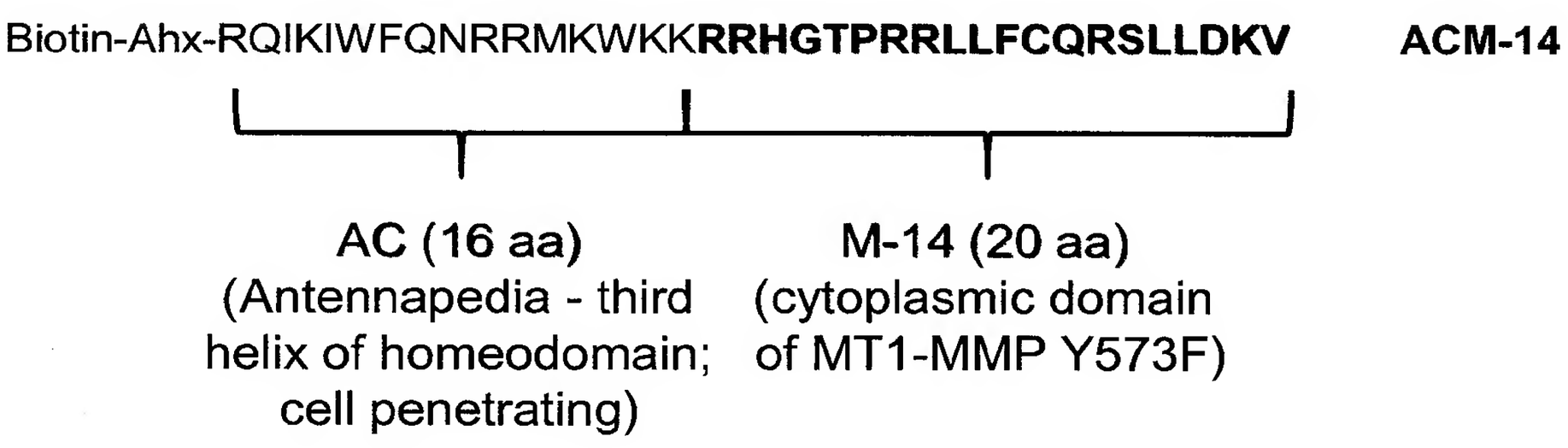

Biotin-Ahx-RQIKIWFQNRRMKWKKRRHGTPRRLLFCQRSLLDKV ACM-14
AC (16 aa)
(Antennapedia - third helix of homeodomain; cell penetrating)
M-14 (20 aa)
(cytoplasmic domain of MT1-MMP Y573F)
Biotin-Ahx-RQIKIWFQNRRMKWKKTLRQRRCLPHFDSGLRKVRL scACM-14

Figure 1

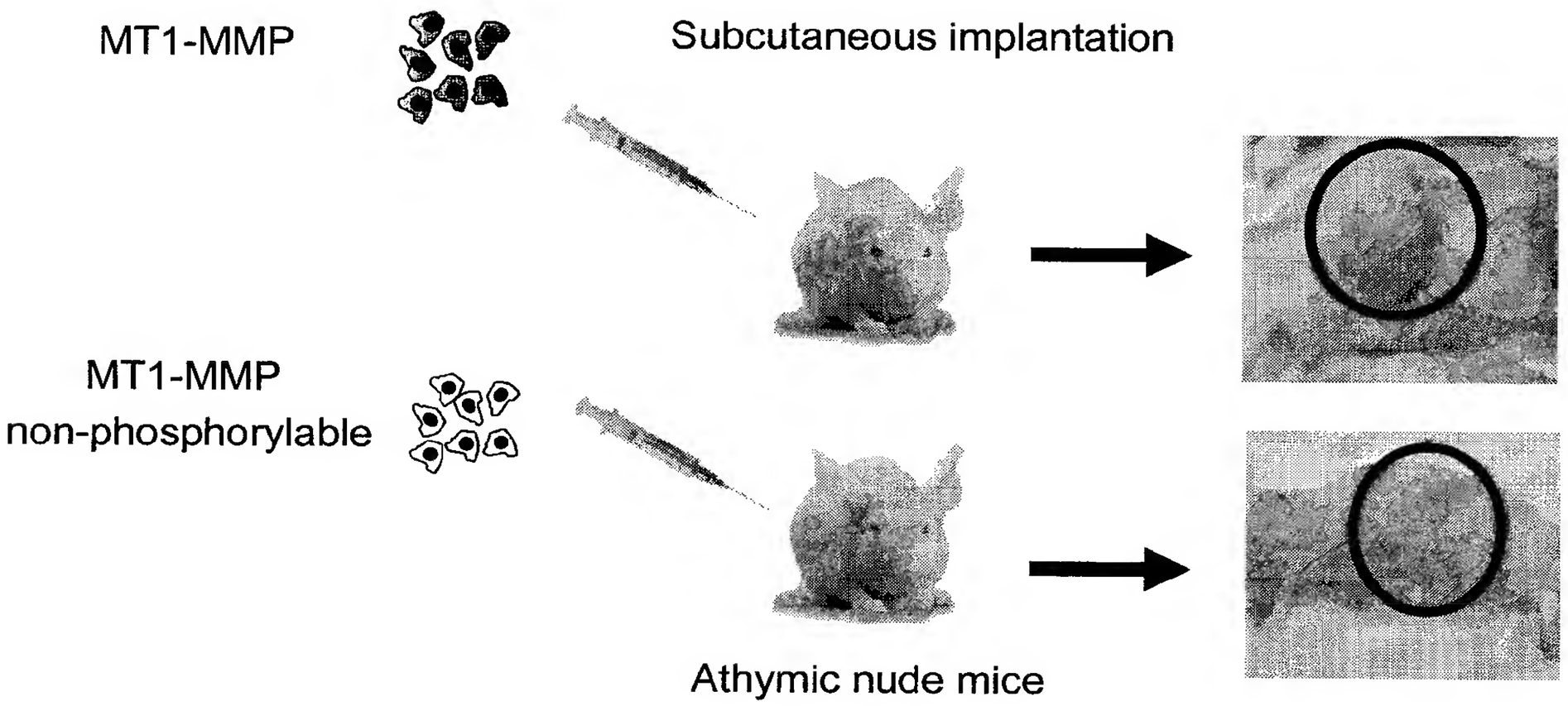

MT1-MMP
Subcutaneous implantation
MT1-MMP
non-phosphorylable
Athymic nude mice

Figure 2

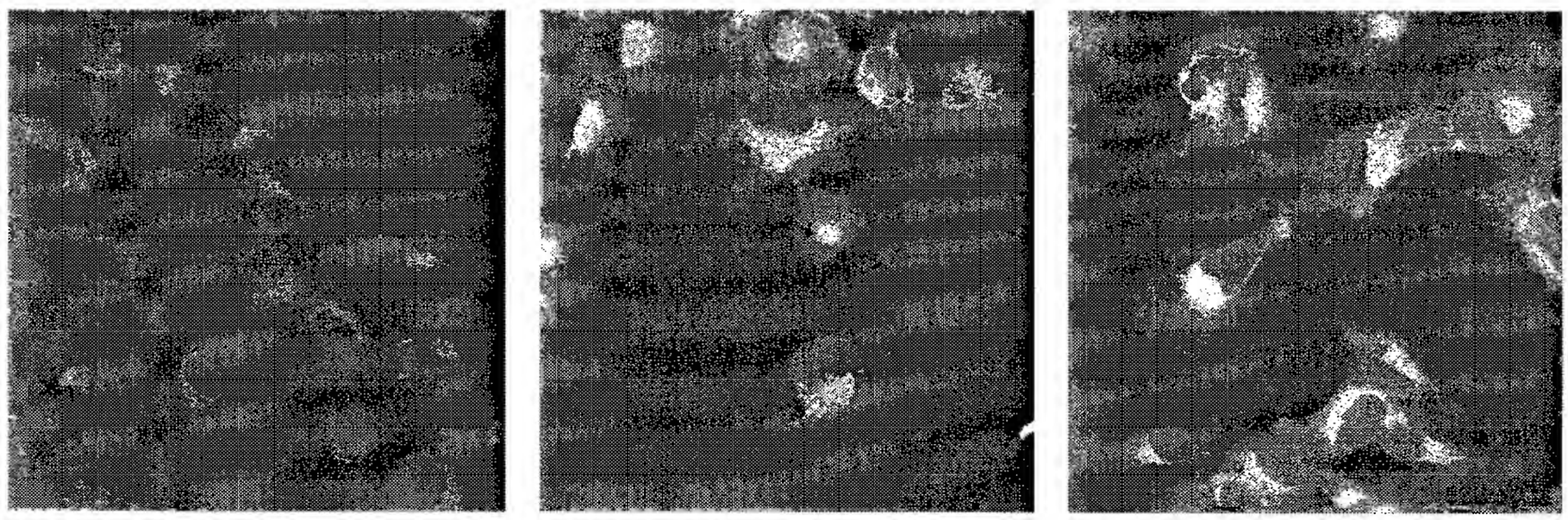


Figure 3

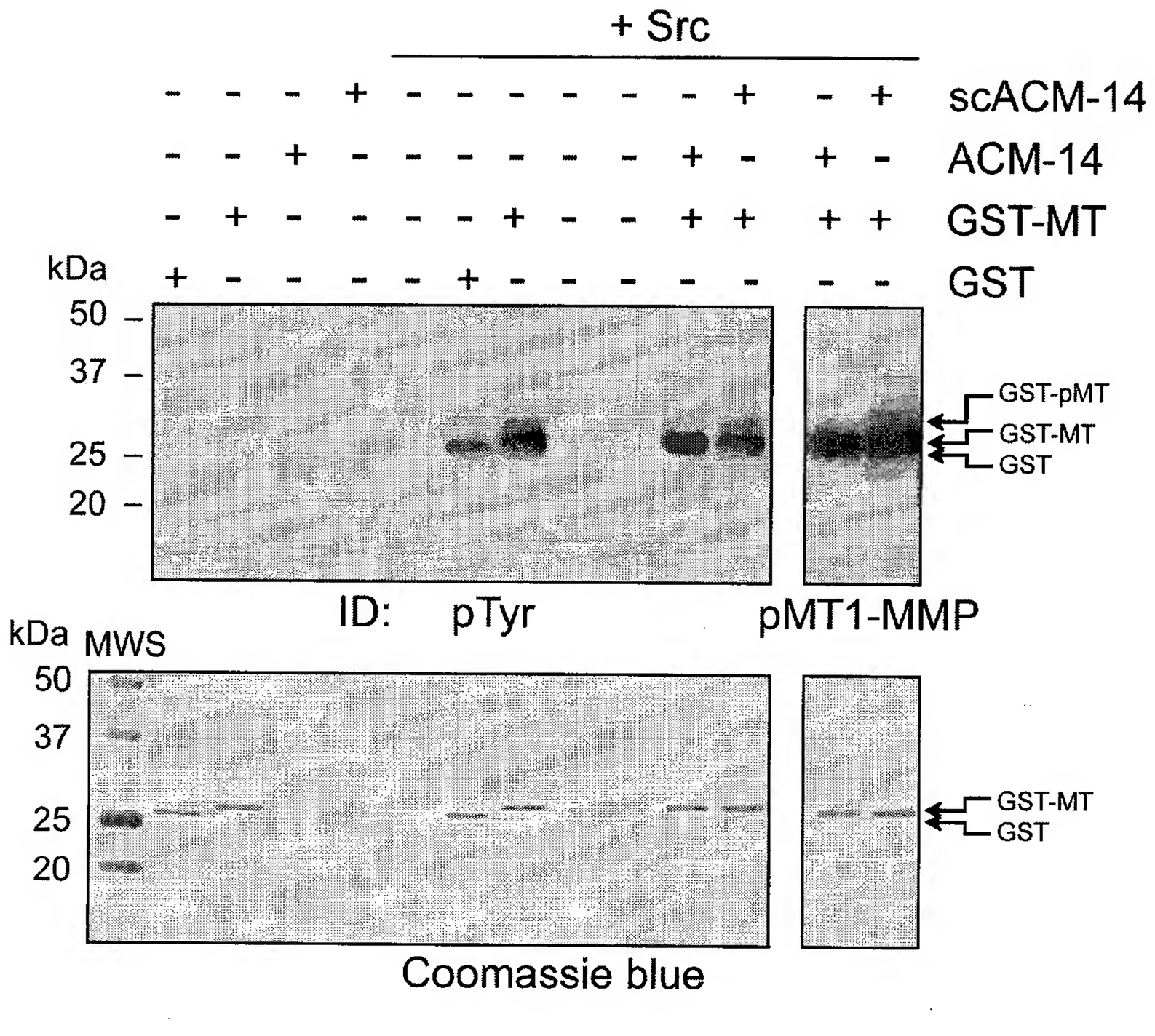
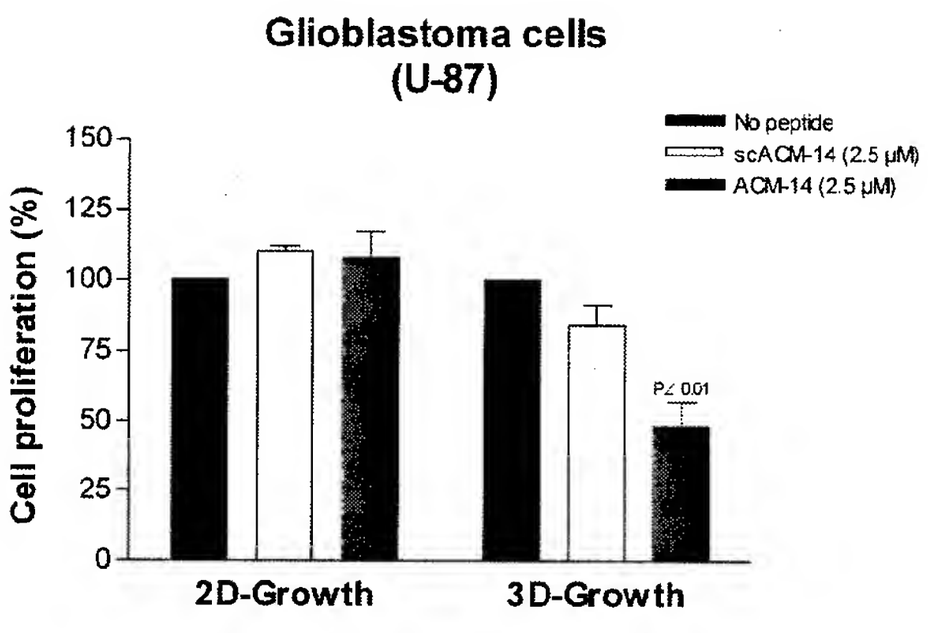
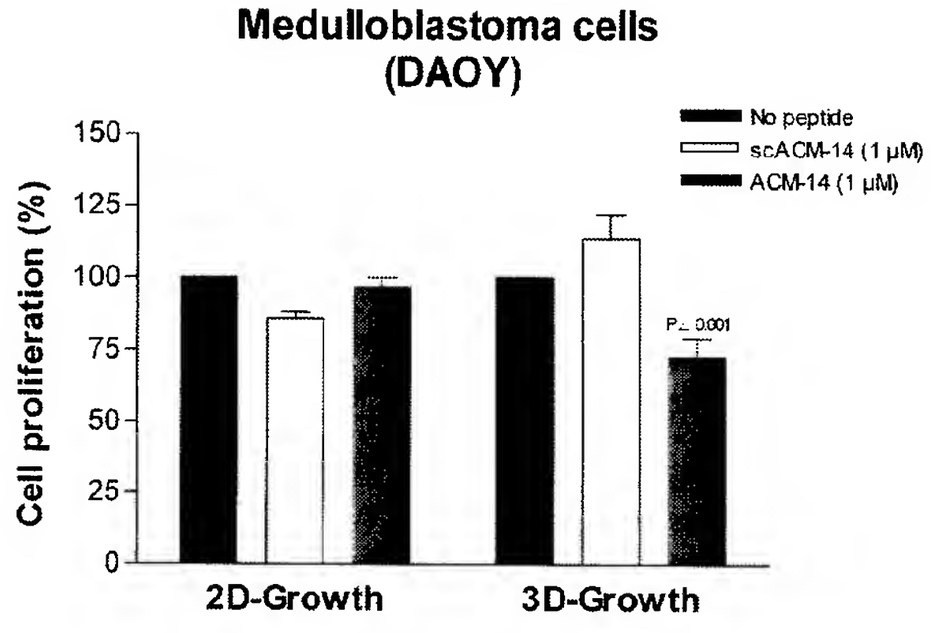
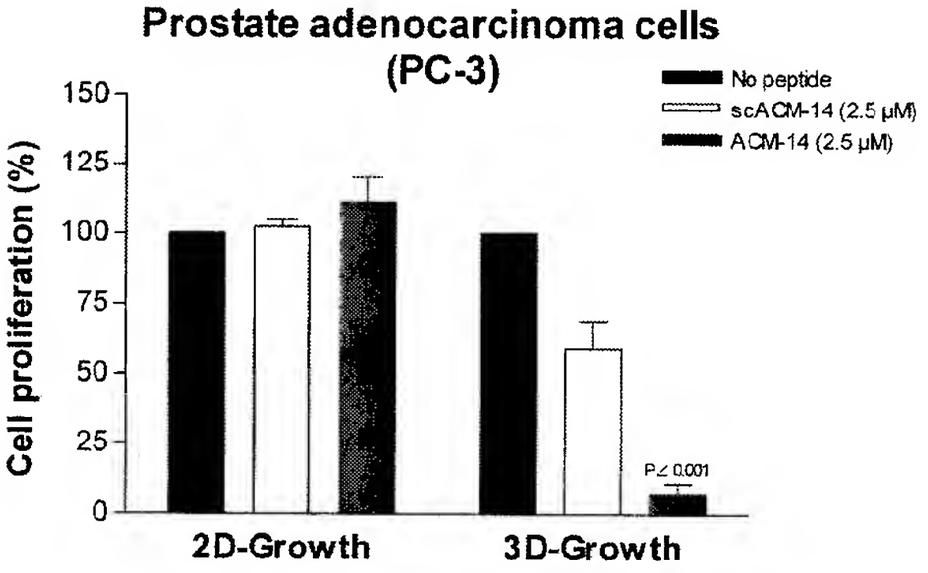
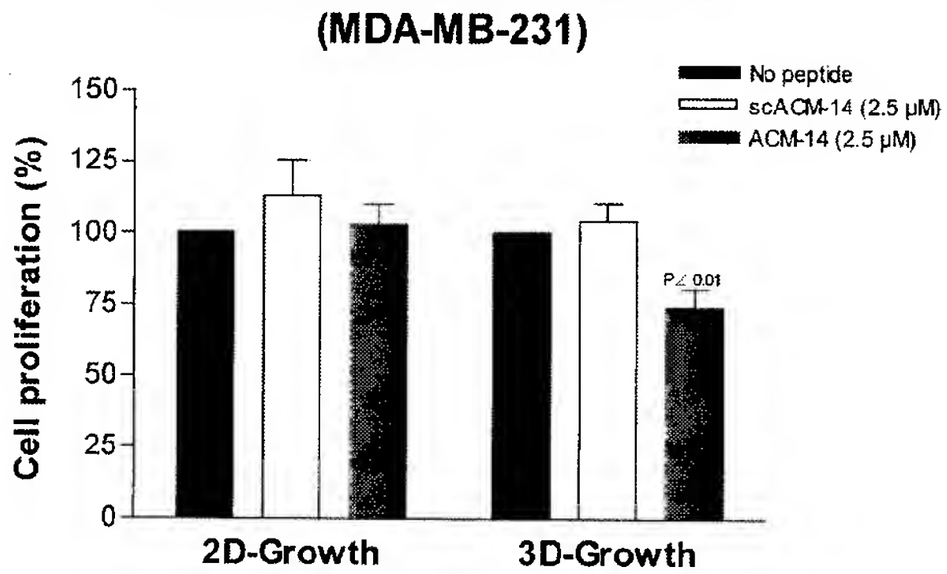

+ Src
- - - + - - - - - - + - + scACM-14
- - + - - - - - - + - + - ACM-14
- + - - - - + - - + + + + GST-MT
kDa + - - - - + - - - - - - - GST
50
37
25
20
GST-pMT
GST-MT
GST
ID: pTyr
pMT1-MMP
kDa MWS
50
37
25
20
GST-MT
GST
Coomassie blue

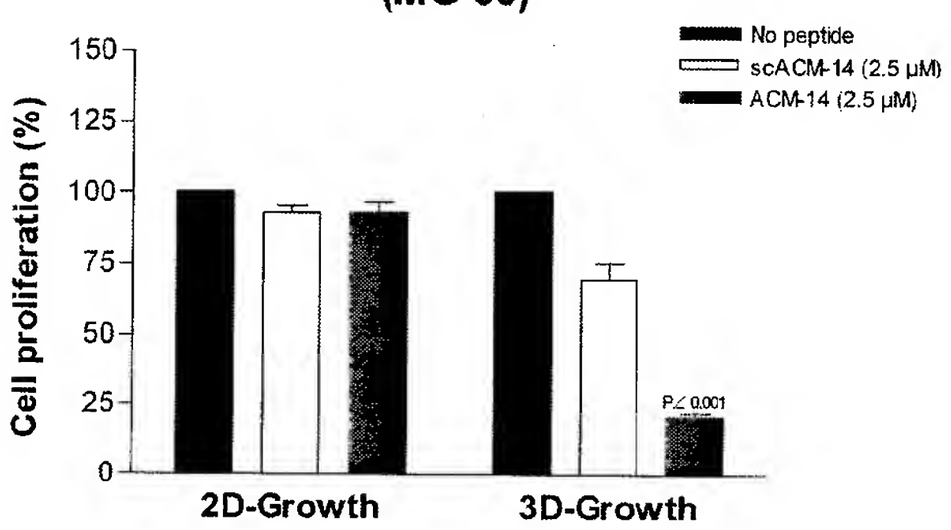
Figure 4


Glioblastoma cells
(U-87)
No peptide
scACM-14 (2.5 µM)
ACM-14 (2.5 µM)
Cell proliferation (%)
150
125
100
75
50
25
0
P< 0.01
2D-Growth
3D-Growth


Medulloblastoma cells
(DAOY)
No peptide
scACM-14 (1 µM)
ACM-14 (1 µM)
Cell proliferation (%)
150
125
100
75
50
25
0
P< 0.001
2D-Growth
3D-Growth


Prostate adenocarcinoma cells
(PC-3)
No peptide
scACM-14 (2.5 µM)
ACM-14 (2.5 µM)
Cell proliferation (%)
150
125
100
75
50
25
0
P< 0.001
2D-Growth
3D-Growth


Breast adenocarcinoma cells
(MDA-MB-231)
No peptide
scACM-14 (2.5 µM)
ACM-14 (2.5 µM)
Cell proliferation (%)
150
125
100
75
50
25
0
P< 0.01
2D-Growth
3D-Growth


Osteosarcoma cells
(MG-63)
No peptide
scACM-14 (2.5 µM)
ACM-14 (2.5 µM)
Cell proliferation (%)
150
125
100
75
50
25
0
P< 0.001
2D-Growth
3D-Growth

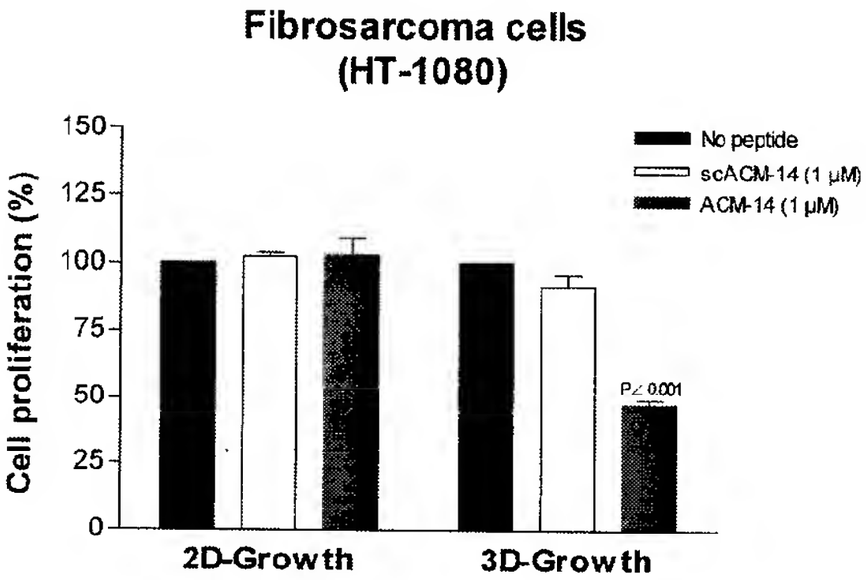
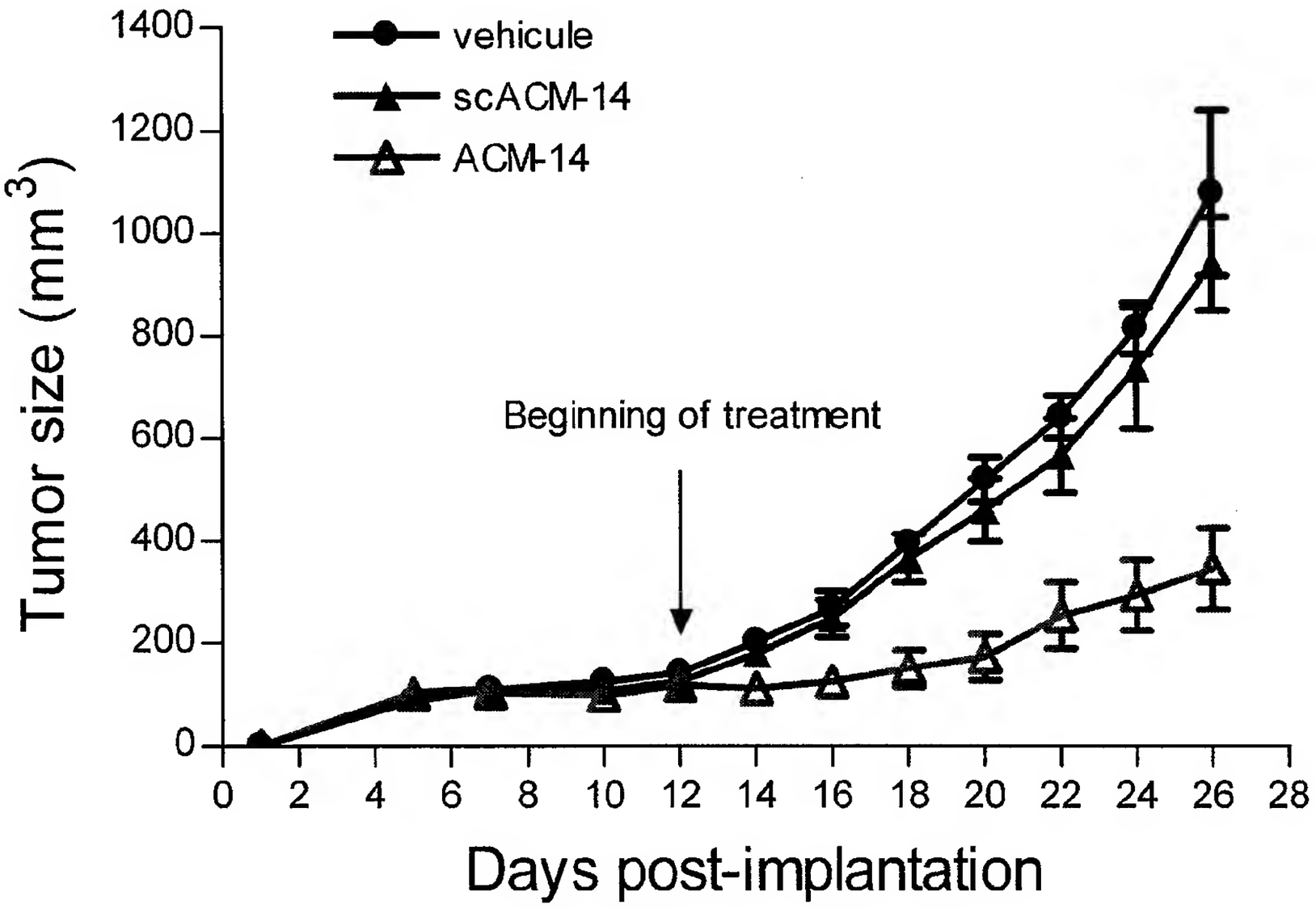
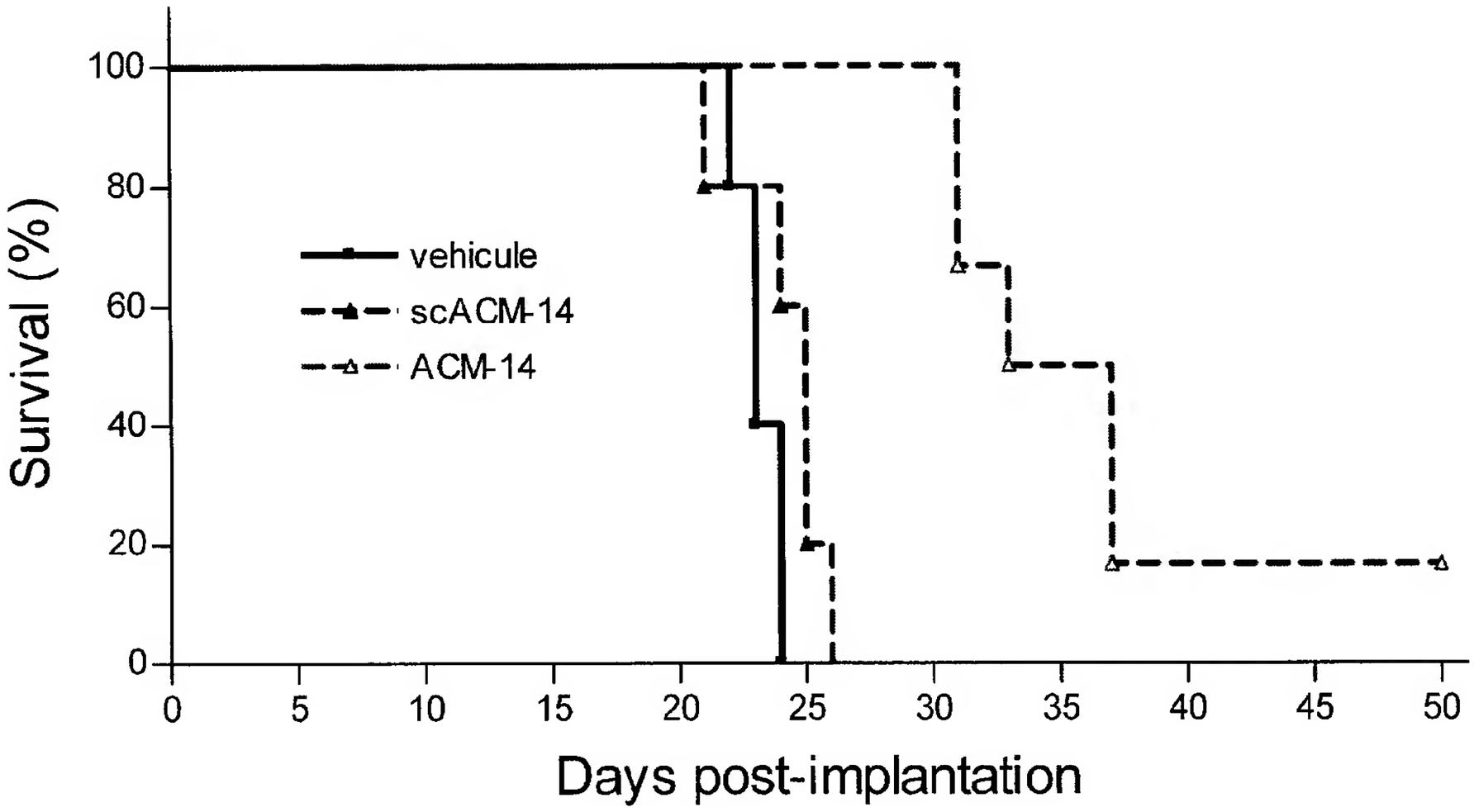

Fibrosarcoma cells
(HT-1080)
No peptide
scACM-14 (1 µM)
ACM-14 (1 µM)
Cell proliferation (%)
150
125
100
75
50
25
0
P< 0.001
2D-Growth
3D-Growth

Figure 5



Dose: 10 mg/kg
Cells: Fibrosarcoma (HT-1080)
scACM-14: scrambled ACM-14

Figure 6



Dose: 10 mg/kg
Cells: Fibrosarcoma (HT-1080)
scACM-14: scrambled ACM-14

Figure 7

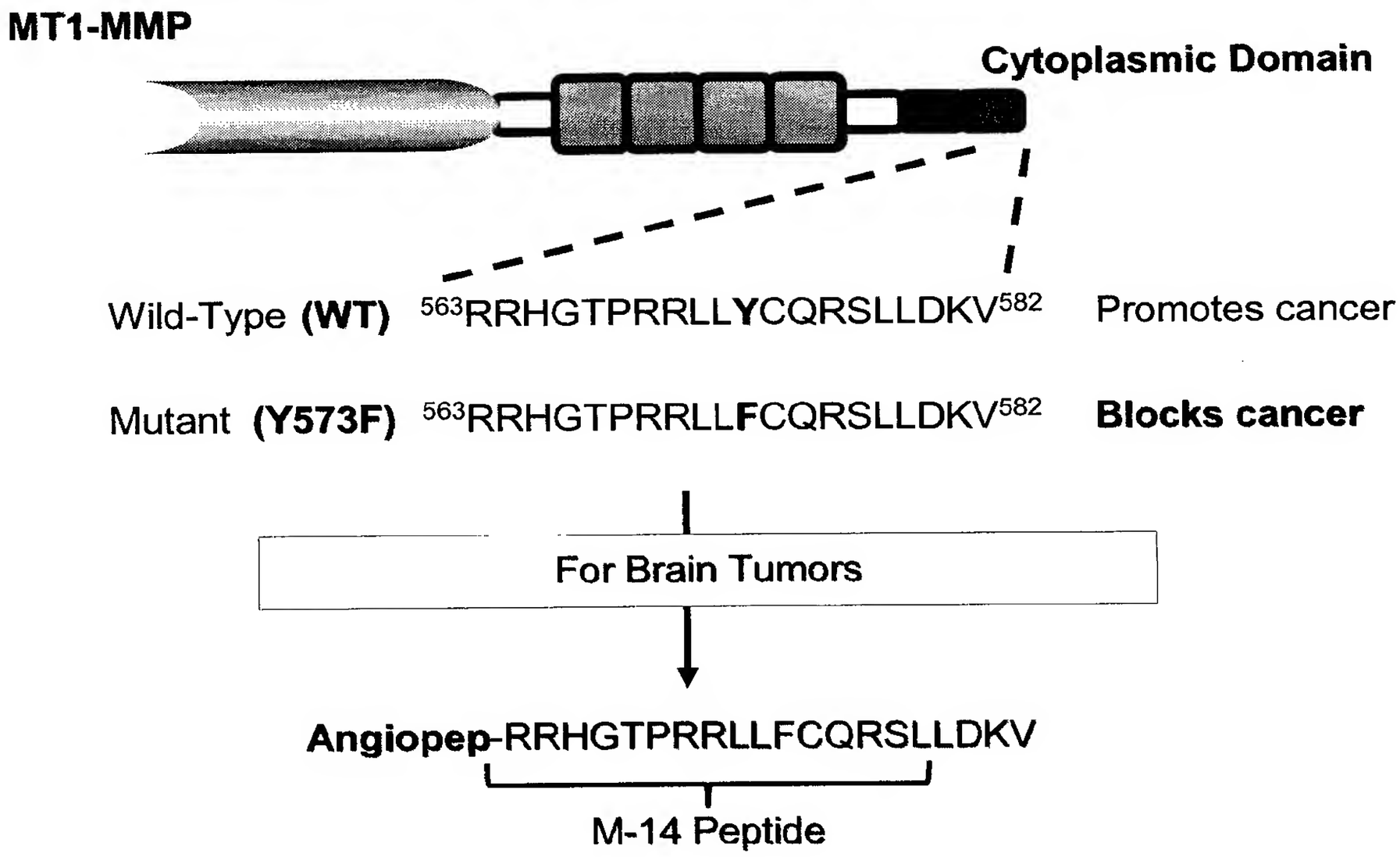

MT1-MMP
Cytoplasmic Domain
Wild-Type (WT) 563RRHGTPRRLLYCQRSLLDKV582 Promotes cancer
Mutant (Y573F) 563RRHGTPRRLLFCQRSLLDKV582 Blocks cancer
For Brain Tumors
Angiopep-RRHGTPRRLLFCQRSLLDKV
M-14 Peptide

Figure 8

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2009/001858

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing filed or furnished:

   a. (means)

   [ ] on paper

   [X] in electronic form

   b. (time)

   [ ] in the international application as filed

   [ ] together with the international application in electronic form

   [X] subsequently to this Authority for the purposes of search

2. [ ] In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments :

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2009/001858

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of the first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons :

1. [X] Claim Nos. : 23-35

because they relate to subject matter not required to be searched by this Authority, namely :

Claims 23-35 are directed to a method for treatment of the human or animal body by surgery or therapy which the International Search Authority is not required to search. However, this Authority has carried out a search based on the alleged effects or purposes/uses of the product defined in claims 1-22.

2. [ ] Claim Nos. :

because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically :

3. [ ] Claim Nos. :

because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows :

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claim Nos. :

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim Nos. :

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

[ ] The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2009/001858

A. CLASSIFICATION OF SUBJECT MATTER
IPC: ***C12N 9/64*** (2006.01), ***A61K 38/10*** (2006.01), ***A61K 38/48*** (2006.01), ***A61K 38/57*** (2006.01), ***A61P 19/02*** (2006.01), ***A61P 35/00*** (2006.01), *C07K 14/81* (2006.01)
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC: *C12N 9/64* (2006.01) , *A61K 38/10* (2006.01) , *A61K 38/48* (2006.01) , *A61K 38/57* (2006.01)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)
GenomeQuest (searched SEQ ID NO:117 and 118), Canadian Patent Database, QPAT, Medline, Scopus, Internet (keywords: membrane type 1 matrix metalloproteinase, MT1-MMP, MMP14, cytoplasm, antennapedia)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	UEKITA, T et al., "Membrane-type 1 matrix metalloproteinase cytoplasmic tail-binding protein-1 is a new member of the Cupin superfamily", Journal of Biological Chemistry. 26 March 2004 (26-03-2004), Vol. 279 pages 12734-12743. ISSN 0021-9258 *Figure 3A*	1, 2, 6, 7, 20-22 9-11, 14, 15, 18, 19
X Y	UEKITA, T et al., "Cytoplasmic tail-dependent internalization of membrane-type 1 matrix metalloproteinase is important for its invasion-promoting activity", Journal of Cell Biology. 24 December 2001 (24-12-2001), Vol. 155 pages 1345-1356. ISSN 0021-9525 *page 1348, right column; Figure 5A; page 1355, left column, third paragraph*	1, 3, 4, 6, 7, 20-22 9-11, 14, 15, 18, 19

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents :
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
5 February 2010 (05-02-2010)	23 March 2010 (23-03-2010)
Name and mailing address of the ISA/CA Canadian Intellectual Property Office Place du Portage I, C114 - 1st Floor, Box PCT 50 Victoria Street Gatineau, Quebec K1A 0C9 Facsimile No.: 001-819-953-2476	Authorized officer Jeremy Mclean (819) 994-9302

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2009/001858

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	TILSTRA, J et al., "Protein transduction: identification, characterization and optimization", Biochemical Society Transactions. August 2007 (08-2007), Vol. 35 pages 811-815 ISSN 0300-5127 *page 811-812, Figure 2*	9-11
Y	WO2008/144919 A1 (BELIVEAU et al.) 4 December 2008 (04-12-2008). *page 1, lines 23-30; page 22, lines 5-20*	9, 14, 15, 18, 19
A	NYALENDO et al., "Impaired tyrosine phosphorylation of membrane type 1-matrix metalloproteinase reduces tumor cell proliferation in three-dimensional matrices and abrogates tumor growth in mice", Carcinogenesis. epub: 10 July 2008 (10-07-2008), Vol. 29, pages 1655-1664. ISSN 0143-3334 *whole document*	1-22

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CA2009/001858

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
WO 2008144919A1	04-12-2008	AU 2005327497A1	24-08-2006
		AU 2006272405A1	25-01-2007
		AU 2008255556A1	04-12-2008
		BR PI0520032A2	14-04-2009
		CA 2597958A1	24-08-2006
		CA 2614687A1	25-01-2007
		CA 2688344A1	04-12-2008
		CN 101160403A	09-04-2008
		CN 101262890A	10-09-2008
		EP 1859041A1	28-11-2007
		EP 1859041A4	08-10-2008
		EP 1907009A1	09-04-2008
		EP 1907009A4	02-09-2009
		JP 2008529539T	07-08-2008
		JP 2009500431T	08-01-2009
		MX 2007010113A	07-12-2007
		RU 2007134566A	27-03-2009
		RU 2008105677A	20-08-2009
		US 7557182B2	07-07-2009
		US 2006189515A1	24-08-2006
		US 2008299039A1	04-12-2008
		US 2009016959A1	15-01-2009
		US 2009082277A1	26-03-2009
		WO 2006086870A1	24-08-2006
		WO 2007009229A1	25-01-2007
		ZA 200706917A	29-10-2008